

BIOLUMINESCENCE

Bioluminescence

By

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PREFACE

The production of light without heat by living things has always appealed to the imagination and excited the interest of mankind. As a remarkable example of functional activity in animals and plants, bioluminescence itself not only presents many problems but has also become an important means of understanding vital processes in general. This is due in part to the development of highly accurate and rapid devices for recording weak lights and in part to a realization that luminescence intensity is a direct measure of the velocity of oxidative enzyme reactions intimately connected with the life of the cell. The light emitted by cells or cell extracts under various conditions can serve as a tool of great value for quantitative biophysical and biochemical investigation. This type of research has already yielded important results in the analysis of enzyme kinetics, drug action, temperature, and pressure effects.

Three types of luminous organisms have been extensively studied—the bacteria, the ostracod crustacean, *Cypridina*, and fire-flies—but they represent only a small proportion of the approximately forty additional groups which contain luminous species. Since many of the other little known luminous forms offer special advantages for chemical and physiological work, the author has collected the facts concerning them and reviewed the present knowledge of all groups of luminous organisms in this volume—a comprehensive monograph of the reference type. It is intended as a complete guide to knowledge on the subject. Biological aspects of light production have been included and the direction for future research suggested.

The compilation has not been an easy task. Fortunately, however, it is neither necessary nor desirable to trace the ideas regarding light emission by living organisms to the beginnings of scientific thought but only to mention the pioneers of the previous century. In this way due credit can be given to discoveries which are often overlooked or for which space is unavailable in the modern technical journal.

The plan of the book is simple. The various luminous groups have been treated in phylogenetic order as biological entities, with a short discussion of the luminous species and their habits, followed by a statement of the known facts concerning morphology, histology, physiology, biochemistry, and biophysics of light producing cells or organs. Where

the results of previous work are too extensive to warrant inclusion, references will be found in the Bibliography.

At one time it was hoped that lists of all known luminous animals in each group might be published, but problems of synonymy and space considerations have prevented carrying out the plan. In referring to luminous organisms, no attempt has been made to bring the nomenclature completely up to date. Whatever the latest scientific name of a particular species may be, it will be designated by the name used in the original publication dealing with its luminescence.

It is a pleasure to acknowledge the advice and help given me in discussion with my former students, especially Frank H. Johnson and Wm. D. McElroy, and my colleagues, Aurin M. Chase and John B. Buck, whose important researches on bioluminescence have done so much to advance the subject. Sincere thanks are due to many who have identified species or advised on systematic matters: Wm. W. Diehl, Edith K. Cash, D. P. Rogers, and Ruth Macrae on Fungi; the late C. A. Kofoid on Protozoa; Elizabeth Deichmann on Cnidaria and Ctenophora; R. S. Bassler on Bryozoa; J. P. Moore and Grace E. Pickford on Annelida; Waldo L. Schmitt, F. A. Chace, Jr., W. L. Tressler, C. R. Shoemaker, the late W. M. Tattersall, and the late C. B. Wilson on Crustacea; R. V. Chamberlin and H. F. Loomis on Myriapoda; E. A. Chapin, G. Vogt, P. J. Darlington, and the late H. S. Barber on Coleoptera; P. Bartsch on Mollusca; G. E. Pickford and W. J. Rees on Cephalopoda; the late H. L. Clark on Echinodermata; W. G. Van Name and N. J. Berrill on Tunicates; J. T. Nichols, A. E. Parr, and T. H. Watermann on Pisces; to those who have lent photographs or drawings, cited in connection with the figures, particularly to the Princeton University Press for permission to reproduce some of the figures of "Living Light"; to the librarians whose aid in finding the older references has been invaluable: Mrs. V. T. Phillips of the Academy of Natural Sciences, Philadelphia, Miss Hazel Gay of the American Museum of Natural History, New York, Mrs. Gertrude Hess of the American Philosophical Society library, Mrs. Deborah Harlow of the Marine Biological Laboratory library, Woods Hole, Mass., and Miss Genevieve Cobb of the Biology library of Princeton University; to my secretary, Mrs. T. M. Page, for valuable assistance in typing manuscript and checking references; and particularly to my wife, Dr. Ethel Browne Harvey, who has aided in translation of foreign languages, read the manuscript, and advised in many ways.

E. NEWTON HARVEY

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INTRODUCTION

Perhaps the most striking biological fact regarding the emission of light by animals and plants is the great number of totally unrelated and diverse organisms which have developed this ability. Although the ratio of number of luminous species to the total number of known species is vanishingly small, the ratio of phyla or classes containing luminous animals to the total recognized phyla or classes is surprisingly large. Exact figures will depend on the classification used, since different zoologists recognize between 10 and 33 phyla. The official American list* of phyla, classes and orders of the animal kingdom represents one extreme. It contains 33 phyla and 80 classes. Among these 13 of the phyla (with two dubious) and 28 of the classes (with 3 dubious) contain luminous species.

The author has followed a classification** intermediate between the two extremes, containing 25 phyla, in 12 or 13 of which are to be found luminous species. This classification, with its 13 luminous phyla and some 28 luminous classes in italics, follows:

Protozoa

Plasmodroma

Flagellata or *Mastigophora* (flagellates)

Rhizopoda or *Sarcodina* (rhizopods)

Sporozoa (sporozoans)

Ciliophora

Ciliata (ciliates)

Suctoria (suctorians)

Mesozoa or *Moruloidea*

Rhombozoa or *Dicyemida* (*Dicyema*)

Orthonectida (*Rhopalura*)

Porifera (sponges)

Calcarea or *Calcispongiae* (calcareous sponges)

Hexactinellida (glass sponges)

Demospongiae (naked or horny sponges)

Cnidaria

Hydrozoa (hydroids)

Scyphozoa (medusae)

Anthozoa (corals, sea pens, etc.)

* "Zoological Names," prepared by A. S. Pearse for Section F, A.A.A.S. Durham, N.C., 1949.

** From "Selected Invertebrate Types," edited by F. A. Brown, Jr., John Wiley Sons, New York, 1950.

Ctenophora (comb-jellies)*Tentaculata**Nuda* (only *Beroidae*)*Platyhelminthes**Turbellaria* (flat worms)*Trematoda* (flukes)*Cestoda* (tape-worms)*Rhynchocoela* or *Nemertinea* (nemerteans)*Acanthocephala* (spiny-headed worms)*Aschelminthes**Rotifera* (rotifers)*Gastrotricha* (*Chaetonotus*)*Kinorhyncha* or *Echinodera* (*Echinoderes*)*Priapulida* (*Priapulius*)*Nematoda* (*Nematodes*)*Nematomorpha* or *Gordiacea* (hair-worms)*Entoprocta* or *Kamptozoa*? *Ectoprocta* or *Polyzoa* (bryozoans)? *Gymnolaemata**Phylactolaemata**Phoronidea* (*Phoronis*)*Annelida**Polychaeta* (marine worms)*Archiannelida* (archiannelids)*Oligochaeta* (earthworms)*Hirudinea* (leeches)*Echiuroidea**Echiurida* (*Echiurus*)*Saccosomatida* (*Saccosoma*)*Sipunculoidea* (*Sipunculus*)*Mollusca**Amphineura* (*Chaetoderma* and *Chiton*)*Scaphopoda* (tooth shells)*Pelecypoda* or *Lamellibranchiata* (clams, etc.)*Gastropoda* (snails)*Cephalopoda* (squid)*Brachiopoda* (lamp shells)*Inarticulata**Articulata**Onychophora* (*Peripatus*)*Arthropoda**Chelicerata**Merostomata* or *Xiphosura*. (*Limulus*)*Pycnogonida* or *Pantopoda* (sea spiders)*Arachnida* (spiders, scorpions, etc.)*Mandibulata**E crustacea* (crustaceans)*Pauropoda* (*Pauropus*)*Diplopoda* (millipedes)*Symphylla* (*Scolopendrella*)

- Chilopoda* (centipedes)
- Insecta* (insects)
- Linguatulida (Linguatula)
- Tardigrada (water bears)
- ? *Chaetognatha* (Sagitta)
- Echinodermata*
 - Eleutherozoa*
 - ? *Asteroidea* (star-fish)
 - Ophiuroidea* (brittle stars)
 - Echinoidea (sea urchins)
 - Holothuroidea (holothurians)
 - Pelmatozoa
 - Crinoidea (crinoids)
- Enteropneusta* or *Hemichorda*
 - Balanoglossida* (Balanoglossus)
 - Cephalodiscida or Pterobranchia (Cephalodiscus)
- Chordata*
 - Tunicata* or *Urochorda* (tunicates)
 - Larvacea* (Appendicularia)
 - Ascidiacea (ascidians)
 - Thaliacea* (Salpa, Pyrosoma)
 - Cephalochorda or Leptocardia (Amphioxus)
 - Vertebrata* or *Craniata* (vertebrates)
 - Cyclostomata (cyclostomes)
 - Pisces* (fish)
 - Amphibia (amphibia)
 - Reptilia (reptiles)
 - Aves (birds)
 - Mammalia (mammals)

In later chapters the author has, unless otherwise specified, used the subdivision into orders, families, and genera given in the "Handbuch der Zoologie" edited by W. Kükenthal and T. Krumbach.

In the plant kingdom, there has been more agreement on large divisions. Most botanists recognize 4 subkingdoms and about 9 divisions. If the dinoflagellates are considered animals, the only luminous plants* are to be found among the bacteria and the higher fungi, which means that a definitely smaller per cent of plant groups have developed the ability to emit light. The distribution in the plant kingdom can be seen from the following simple scheme in which the groups containing luminous species are italicized.

Thallophyta

Algae

- Cyanophyceae (Blue-green Algae)
- Chlorophyceae (Green Algae)

* Recently Strehler and Arnold (1951) have described chemiluminescent light emission of very low intensity accompanying photosynthetic activity.

Phaeophyceae (Brown Algae)

Rhodophyceae (Red Algae)

Fungi

Myxomycetes (Slime moulds)

Schizomycetes (Bacteria)

Phycomycetes (moulds)

? *Ascomycetes* (Sac fungi, yeasts, some moulds)

Basidiomycetes (Smuts, rusts, mushrooms)

Bryophyta

Hepaticae (Liverworts)

Musci (Mosses)

Pteridophyta

Equisetineae (Horsetails)

Lycopodineae (Club Mosses)

Filicineae (Ferns)

Spermatophyta

Gymnospermae (Cycads, Ginkgo, Conifers)

Angiospermae (Mono- and Dicotyledonous flowering plants)

It is apparent from the previous classifications that no clear development of luminosity along evolutionary lines is to be detected but rather a cropping up of luminescence here and there, as if a handful of damp sand has been cast over the names of various groups written on a black-board, with luminous species appearing wherever a mass of sand struck. The Ctenophora have received the most sand. It is probable that all members of this phylum are luminous. The Cnidaria also contain many luminous species scattered among certain of the orders.

At the other extreme are very large groups in which only a few luminous animals are known, as in the gastropod and lamellibranch molluscs. It is an extraordinary fact that one species in a genus may be luminous and another closely allied species may contain no trace of luminosity. Only among animals with complicated luminous organs or photophores, such as shrimp, squid, and fish, does there appear to be a definite series of gradations with increasing complexity, that might be regarded as an evolutionary line. Elsewhere the ability to emit light must have arisen independently in widely scattered groups.

Another striking peculiarity of luminescence distribution is the almost complete absence of luminous species in fresh water. The most striking instance of this rule is to be found among dinoflagellates in which only the salt water species can emit light. The only true exception known at the present time is the fresh water lumpet, *Latia*, of New Zealand. Possibly aquatic fire fly larvae of inland pools and streams and fresh water luminous bacteria, which sometimes grow within living fresh water shrimp can also be called exceptions.

Although luminous species are abundant in the depths of the ocean,

they do not occur in the depths of inland lakes or in the fresh water of caves. Parasitic luminous species (except bacteria) are also unknown, and among terrestrial animals luminosity cannot be connected with any peculiarity of habitat or relationship, except that luminous forms are **almost universally nocturnal**.

There is no doubt whatever but that bioluminescence is a form of chemiluminescence in which definite chemical substances emit the light during a chemical reaction. The process can be completely imitated by organic compounds of known composition in the laboratory. Luciferin and luciferase are general names used for these compounds manufactured by luminous animals, but it is probable that the luciferin or luciferase from a species in one group may be quite different chemically **from that in another**.

The light emission of living things may be intracellular or appear only after the luminous materials have been secreted to the exterior (extracellular luminescence). The fine structure of the luminous cells or luminous organs may be very different in different groups. In some cases the luminous organ may be so complicated by accessory structures, like lenses, reflectors, or color screens, that the whole makes up a veritable lantern. Detailed information on all aspects of the light-emitting process in various groups of animals and plants will be found **in the following pages**.

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CHAPTER I

Bacteria

SAPROPHYTIC LUMINOUS SPECIES

Whenever dead fish, flesh of animals of all kinds including man, eggs, sausages, and various dead invertebrates become luminous, it is practically certain that the light results from the growth of luminous bacteria. If the luminous material is dead vegetable matter, wood, roots, beets, potatoes,¹ fruit,² etc., the light is usually due to luminous fungi. Phosphorescence of flesh was known to Aristotle and has aroused interest and curiosity among the learned from the sixteenth century on. The history of attempts to explain the origin of the light makes fascinating reading, but space limitations allow only a few important discoveries to be recorded here.

Possibly the first hint that phosphorescence of dead fish and flesh might be due to living things is due to Baker in 1742. In his book, *The Microscope Made Easy*, he suggested that the light might come from "animacules," just as he had found the light of the sea to be due to tiny "insects."

Many of the fundamental discoveries regarding the properties of bacterial light were made by men who did not know that living matter was involved. Boyle, using his air pump in 1668, noted the dependence of the light of both wood and fish on the air and hence on oxygen, although oxygen as an entity was not known at the time. Martin, in 1761, discovered the necessity of salt for marine luminous bacteria although he thought he was studying phosphorescence of the sea. Canton in 1769, during his study of luminous fish, was the first to observe the reversible extinction of the light of bacteria by rise in temperature, although he little realized the importance that this effect was

¹ Cooked potatoes sometimes serve as culture media for luminous bacteria (see Molisch, 1904, 12) and luminous cheese may be due to luminous fungi, but the actual cause has not been determined.

² Gobbel (1824) reported luminescence of bubbles of CO₂ accompanying the fermentation of raspberry juice, a phenomenon possibly due to luminous bacteria.

to assume in modern studies on the kinetics of light production. Canton wrote, "putting a very small piece of a luminous fish into a thin glass ball, I found that water of the heat of 118 degrees would destroy its luminousness in less than half a minute; which on taking it out of the water, it would begin to recover in about ten seconds, but was never so bright as before." Other names associated with early observation of bacterial luminescence are Cardanus, Fabricius, Borelli, Bartolin, Beale, and Rede. The prize essays of Dartous de Mairan in 1717 and Cohaussen also in 1717, the book of Priestley in 1772, and later prize essays of Bernoulli (1803), Link (1808), Heinrich (1808, 1820), and Dessaignes (1809) all speculate on what was then known concerning the light of fish and flesh.

In the late eighteenth and early nineteenth centuries there were further observations (Delius in 1784; Hulme, 1800, 91; Deslongchamps, 1838) on dead fish, flesh, and a number of experiments on wood to explain the origin of the light. Most of them considered it to be connected with decay and decomposition, more specifically to combustion of some organic compound similar to phosphine. It was Heller in 1853 who definitely named an organism, *Sarcina lutea*, as the cause of the light of meat. Heller showed that new flesh could be inoculated with the luminous material and Pflüger in 1875 demonstrated that the bacteria from fish could be filtered off and would grow on culture media. He did not give them a name, but the omission was rectified by Cohn (1878) who proposed the name, *Micrococcus phosphoreus*.

It is interesting to note that a number of workers (Phipson, 1860, 70; Mulder, 1860; Hankel, 1862; Horne, 1869) between 1853 and 1875 were still unaware of the living nature of the luminescence of dead fish and flesh. One of these was Panceri (1871), who, despite his great contributions to knowledge of many luminous invertebrates, regarded the light from a dead fish, *Trachopterus iris*, which he studied, as due to oxidation of fat.

Subsequent to Pflüger's paper, publications were largely concerned with naming the bacteria. Nuesch (1877, 79) described *Bacterium lucens* and *B. termo*, while Bancel and Husson (1879) found two varieties of bacteria growing on lobsters which they thought produced "carburetted and phosphoretted hydrogen" responsible for the light. Lassar (1880) confirmed Pflüger's work and spoke for micrococci which he considered responsible for the luminescence of dead invertebrates.

*Cooper and Cooper (1838) found that luminous material on human cadavers would cause other non-luminous cadavers to become luminous when rubbed over them.

An early experimental investigation was that of Ludwig (1882, 1884) who described *Micrococcus Pflügeri* and many of its characteristics, including its spectrum. The light appeared pale green, extending from the Fraunhofer line b into the violet. Luminous bacteria had become of great interest to the bacteriological and biological world. Ludwig (1887, 92) continued the study of photogenic bacteria and was followed by a number of workers, Dubois (1885), Fischer (1886, 87, 88), Hermes and Forster (1887), Tilanus (1888-90), and a review by Duclaux (1887).

The most comprehensive study of species was by Fischer, a ship's medical officer, and an early worker in the whole field of marine bacteriology. He discovered *Bacterium phosphorescens* from the West Indies and another species from the Baltic Sea and later (1894) isolated nine new species while on the Humboldt-Plankton expedition, and studied not only cultural characteristics but the general properties of these forms. He was followed by Forster (1887, 92) in Amsterdam, who again observed the spectrum and also noted a slow growth even at the freezing point. Lehmann (1889) and Tollhausen (1889) continued the work on *Bacterium phosphorescens* of Fischer. In the meantime Katz (1887, 91) described in detail in a long paper the characteristics of *Bacillus cyaneo-phosphorescens*, *B. smaragdino-phosphorescens*, *B. argenteo-phosphorescens* I, II, and III and *B. argenteo-phosphorescens liquefaciens*, all from Australia. It is apparent that luminous bacteria are of worldwide distribution.

Dubois (1888-93, 1919) studied *Photobacterium sarcophilum*, *Bacterium pholas* and *B. pelagia*. Beijerinck (1889, 91) *Photobacterium luminosum*, *Ph. indicum*, *Ph. fischeri*, now called *Achromobacter fischeri*, and *Ph. phosphorescens* = *Ph. pflügeri*. Giard (1889, 90) and Giard and Billet (1889, 90) discovered a *Diplobacterium* (later called *Bacterium giardi*) infecting sand fleas, and Eijkmann (1892) isolated *Photobacterium javanense* from fish in Java. One of the most interesting and important discoveries was made by Kutscher (1893, 95) who found a luminous *Vibrio* from the river Elbe, similar to the cholera vibrio, thus establishing the existence of fresh water species. Weleminsky (1895) actually isolated luminous *Vibrios* from patients with Asiatic cholera. The strain was not luminous at first but became luminous after passage through pigeon's blood.

Review or general papers were published by Dubois (1889), Héricourt (1890), Cotton (1891), Clautrian (1896), Migula (1897), Barnard (1899, 1902), and A. Fischer (1900). Barnard's paper is particularly complete, listing 26 different species described up to 1899, giving his own experience with the properties of 12 of them and describ-

ing a new organism, *Photobacterium liquifaciens Plymouthii*. Barnard expressed the belief that many of the bacteria previously described were the same or very similar organisms and emphasized the fact that luminescent bacteria tend to undergo involution and exhibit pleomorphism "presenting at one time a rod shape and at another time assuming the form of a spirillum, whilst mixed forms are not infrequent."

At the beginning of the nineteenth century, Molisch (1902-1905, 12, 25) started his experiments on luminous bacteria from various sources, hen's eggs,¹ sausages, the water of Trieste harbor—which are summarized in the two editions (1904, 12) of his book, *Leuchtende Pflanzen*. He listed 26 well-described species of luminous bacteria in the 1904 edition and 30 in the 1912 edition. About the same time new bacteria were discovered by Tarchanoff (1901), Nadson (1903, 08), Foa and Chiapella (1903), Immaura (1904), Gorham (1904), Ugloff (1908), and particularly Reinalt (1906), who made a careful study of the cultural differences of many different types. Issatschenko (1911) found *Bacterium hippanis* on fresh water fish from the southern Bug River and Beijerinck (1912-15) continued his work on various forms, paying particular attention to the development of new strains. It is apparent that a very extensive variety of luminous bacteria was known early in the twentieth century.

It is not possible to list all the new species of luminous bacteria described in later years. Many of these bacteria have been parasitic on or symbiotic with various animals, making the host luminous while living. These will be considered in special sections. A few bacteria have been obtained from the flesh of dead animals or from fresh or salt water. Among them may be mentioned *Coccobacillus* sp. of Fejgin (1926), *Pseudomonas luminescens*, *P. photogena*, and *P. phosphorescens* of Kishitani (1928, 33), forms from the Black Sea and Sea of Azof (Egorowa, 1929), *Bacterium photodoticum* of Vouk, Skoric, and Klas (1931), *Vibrio phosphorescens* of Maslennikowa (1927), and Stutzer (1929), *V. albensis* of Sonnenschein (1931, 32), new species described by Fuhrmann (1932), and *Achromobacter harveyi* of Johnson and Shunk (1936). Yasaki and Nomura (1947) described luminous forms from the digestive organs of fish, and Baylor² has isolated a number of species with unusual properties from the alimentary tract of deep sea fish obtained at Bermuda. Many observa-

¹ Molisch (1905) has made a special study of the reported cases of bird and reptile egg luminescence and particularly of cooked eggs which are the result of luminous bacterial infection. Haga (1942) in Japanese, has found the penetration of luminous bacteria through the shell but not the membrane of the hen's egg.

² Private communication.

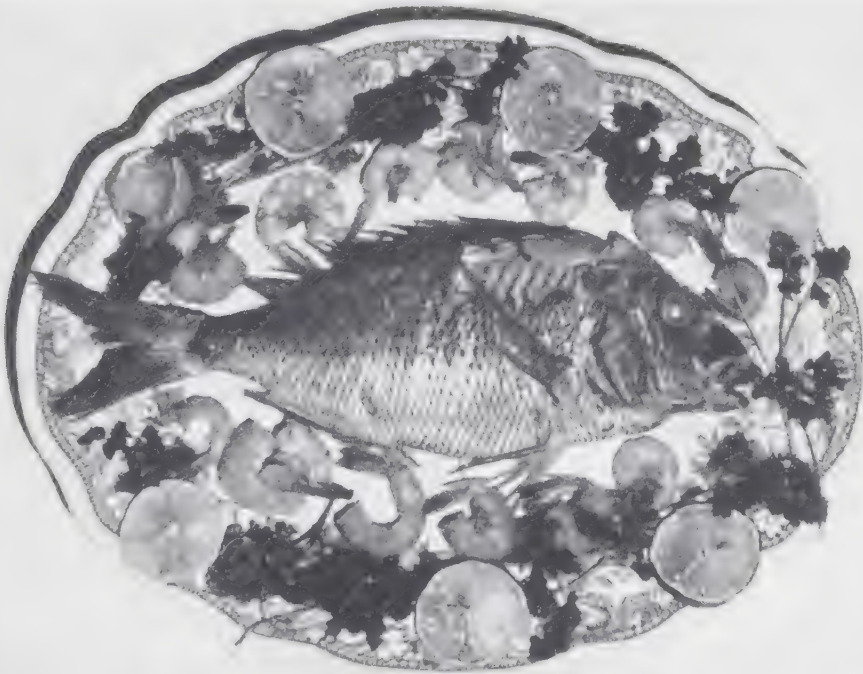
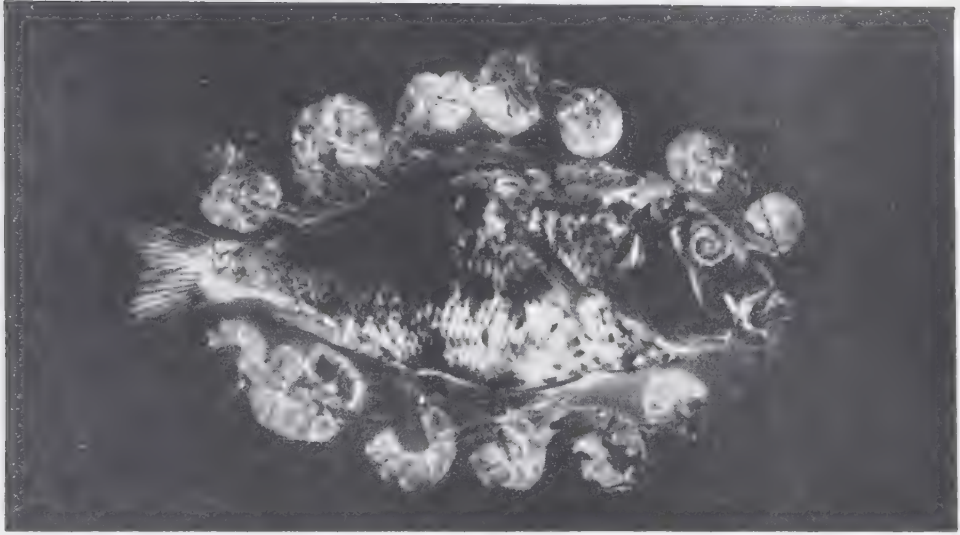


FIG. 1. A dead fish and shrimp infected with luminous bacteria on a plate, photographed by their own light (above) and by daylight (below). Note that lemon slices and parsley are not luminous. Bacteria grown by F. H. Johnson. Courtesy LIFE, (c) TIME Inc.

nous of dead luminous fish and meat have appeared from time to time (Perroncito, 1898, 1904; Prashad, 1923). A photograph of such fish is reproduced in Fig. 1 and the cultures in Fig. 2.

The literature on luminous bacteria is enormous, second only to that on the fire-fly. The pioneers were largely concerned with cultural characteristics. Later, various schools of study appeared whose interests were broader and involved the biochemical and physiological approach. Among the leaders may be mentioned Beijerinck at Delft, Holland; Molisch in Vienna, Austria; Harvey and Johnson in Princeton, N. J.; Kluver and associates at Delft, and Yasaki and students in Japan. The relation of luminous bacteria to living organisms has always excited interest, and special studies in this field have been made by the Pierantoni school in Naples, Italy, and Kishitani and his colleagues in Tokyo, Japan.



FIG. 2. Luminous bacterial colonies growing on culture media in a petri dish, flask, and test tube. After Molisch.

There are very few general scientific publications on luminous bacteria of a comprehensive nature. The book of Molisch (1904, 12) and his review in Lafar's *Handbuch der technischen Mykologie* (1907) are exceptions. Most of the information is in original papers but an excellent review of the chemical aspects of light production will be found in Johnson's (1947) *Bacterial Luminescence*. General articles on bioluminescence devote considerable attention to luminous bacteria and more or less popular accounts have been written by Anon. (1903), Bruini (1906), Reitz (1909), Van de Rovaart (1910); Lafar (1910), Harvey (1926), Hill and Shoup (1929), Shoup (1930), Schubert (1934), Yasaki (1934), Pierantoni (1936), De Lerma (1937, 42), Mortara (1942), Johnson (1948), and Johnson and Eyring (1948).

CLASSIFICATION

Luminous bacteria may be classified as parasitic, causing infection of various living animals (amphipods, insects, or fresh water shrimp

or saprophytic, living on dead organic matter (dead fish or meat), or symbiotic, *always* found in the luminous organs of normal fish or squid. They may be isolated from all these sources and will live in artificial culture media made up with sea water or dilute saline or fresh water, forming the basis for classification into salt water and fresh water forms. In morphology they may be grouped as long or short rods or cocci or vibrios and they may be motile or non-motile. From a chemical standpoint they may be gram positive or negative, may or may not liquefy gelatin or ferment various sugars. The growth characteristics may differ on various media, and they are subject to rather remarkable changes of form. The pH and temperature optima differ in different species, and the wave length of maximum light emission also varies slightly. Finally, the various strains or species exhibit specific immune reactions and some varieties are subject to attack by phage.

The systematic classification of bacteria is a difficult subject and there is considerable disagreement regarding the fundamental characters by which groups should be separated. Probably chemical processes are most important, and at first thought photogenic forms might be grouped together on this basis, but actually the emission of light is no more fundamental than the production of pigment. Like pigment formation, luminescence is easily lost and strains of non-luminous bacterial mutants readily obtained from luminous forms.

Despite the extensive investigation of cultural characteristics, the separation of luminous bacterial species is in a rather unsatisfactory state. Migula (1897) listed 25 well-described luminous species of *Photobacterium*, *Bacterium*, and *Bacillus*, which he later grouped in the genera *Bacillus*, *Bacterium*, *Micrococcus*, *Pseudomonas*, and *Micropsira*. In the first and second editions (1923, 25) of Bergey's Manual of Determinative Bacteriology no mention is made of luminous bacteria, but in the third (1930), fourth (1934), and fifth (1939)⁶ editions four genera are included. In the fifth edition the great class of Schizomycetes is divided into seven orders and 26 families, based on decisions of nomenclature adopted by International Congresses of Microbiology. Luminous bacteria all belong in one order, the Eubacteriales. The families are the Pseudomonadaceae with *Vibrio* and *Pseudomonas*, the Micrococcaceae containing *Micrococcus*, and the Bacteriaceae containing *Achromobacter*.

In the sixth edition (1948) of Bergey, the same seven orders are recognized, but the families have increased to 42. Three luminous genera and seven species are considered definite. These are *Bacterium*

⁶ Manual of Determinative Bacteriology by D. H. Bergey, R. S. Breed, E. G. D. Murray, and A. P. Hitchens, 5th ed., 1939, 6th ed., 1948, Baltimore, Md.

phosphoreum (Cohn) Molisch, *B. phosphorescens indigenus* (Eisenberg) Chester and *B. hemophosphoreum* Pfeiffer and Stammer, *Pseudomonas harveyi* Johnson and Schunk, *P. phosphorescens* (Fischer) Bergey *et al.*, *P. pierantonii* (Zirpolo) Bergey *et al.*, *Vibrio pierantonii* (Zirpolo) Meissner. Forty-five additional species are regarded as inadequately described. They have been placed by their discoverers in various genera, such as *Achromobacter*, *Bacillus*, *Vibrio*, *Photobacterium*, *Photobacter*, *Micrococcus*, *Microspira*, *Arthrobacterium*, *Coccobacillus*, *Photospirillum*, *Pseudomonas*, and *Sarcina*. The synonymy and identification of luminous bacteria are so confused that in this book the various species of luminous bacteria will be referred to by the name used in the particular paper under consideration.

The Bergey latest classification (6th edition)¹ is as follows, with luminous genera printed in italics:

Schizomycetes

Eubacteriales

Eubacteriineae

Nitrobacteriaceae (9 genera including *Nitrobacter*, *Hydrogenomonas*, and *Thiobacillus*)

Pseudomonadaceae (12 genera including *Pseudomonas*, *Acetobacter*, *Vibrio*, *Thiospira*, and *Spirillum*)

Azobacteriaceae (*Azotobacter*)

Rhizobiaceae (*Agrobacterium*, *Rhizobium*, *Chromobacterium*)

Micrococcaceae (*Micrococcus*,[†] *Gaffkya*, *Sarcina*)

Neisseriaceae (*Neisseria*, *Veillonella*)

Lactobacteriaceae (7 genera including *Lactobacillus*, *Diplococcus*, and *Streptococcus*)

Corynebacteriaceae (*Corynebacterium*, *Listeria*, *Erisipelothrix*)

Achromobacteriaceae (*Alkigenes*, *Achromobacter*,[†] *Flavobacterium*)

Enterobacteriaceae (8 genera, including *Escherichia* and *Salmonella*)

Parvobacteriaceae (10 genera, including *Pasteurella* and *Brucella*)

Bacteriaceae (*Bacterium* with subgenera *Kurthia*, *Cellulomonas*, *Saccharobacterium*, *Agarbacterium*, *Photobacterium*, *Methanobacterium*)

Bacillaceae (*Bacillus*, *Clostridium*)

Caulobacteriineae (5 families)

Rhodobacteriineae (3 families)

Actinomycetales (3 families)

Chlamydobacteriales (8 families)

Myxobacteriales (5 families)

Spirochaetales (2 families)

Rickettsiales (3 families)

Virales (phage and virus, 14 families)

[†] The luminous *Micrococcus phosphoreum* is considered a synonym for *Bacterium phosphoreum*, and the genus *Micrococcus* is reserved for non-luminous forms. *Achromobacter* is also considered a non-luminous genus and *A. fischeri* is called *Bacterium phosphorescens indigenus*.

PARASITIC LUMINOUS SPECIES

Although it is convenient to speak of saprophytic, parasitic, and symbiotic luminous bacteria, there are no fundamental differences in the bacteria themselves. Probably the first observation of infection of a living animal must be ascribed to Thulis and Bernard (1786) who described a luminous "Crevette de Rivière" (shrimp) from a fresh water stream in southern France, without knowing that the light must have been due to luminous bacteria. The next instance appears to be that of Viviani (1805), who figured luminous species of what he called *Gammarus* from the Mediterranean near Genoa. Judging from the drawings, the animals were beach fleas.

These papers have been mostly overlooked, and the discovery of a luminous bacterial malady of living animals is usually attributed to Giard (1889) and Giard and Billet (1889, 90). They demonstrated very clearly that sand-fleas of the genera *Talitrus* and *Orchestia* become infected and luminous, live a few days, and then die. These men cultured the bacteria, which they identified as a *Diplobacterium*, later spoken of as *Bacterium giardi*, and showed that the infection could be transferred to other beach fleas and other genera of amphipods and crabs, making them luminous. Russell (1892) also inoculated the shrimp, *Palaemon*, with this bacillus. Beach fleas seem to be particularly susceptible to infection, as the author has observed them at Woods Hole, Mass., where the bacteria were isolated as luminous colonies and studied by Inman (1927). Skowron (1926) succeeded in making many marine animals luminous by injecting ordinary luminous bacteria into them.

Infection of fresh water forms has been investigated by Yasaki (1927) who attributed the discovery of the luminous fresh water shrimp (*Xiphocaridina compressa*) of inland Lake Suwa, Japan, to a Mr. Ushiyama in 1914. The infected shrimp live only a short time but are brilliantly luminous. The infecting organism was isolated and called *Microspira phosphoreum*. A detailed study of its metabolism and immune properties has been made by Majima (1931) under the name of *Vibrio yasakii*.

Among land animals, insects frequently become infected with luminous bacteria. The first case was described by Hablitzl in 1782 among midges of the Bay of Astrabad, Persia. They have been noticed in Russia and other parts of Europe by many persons (Alenitzin, 1875; Kusnezoff, 1890; Schmidt, 1894; Henneberg, 1899; Issatschenko, 1911; Behning, 1929). Issatschenko isolated and named the organism *Bacterium chironomi*.

Caterpillars are very prone to become infected and have been described by Gimmerthal (1829), Boisdual (1832), Rye (1878), Holyrode (1916), Stammer (1930), and Pfeiffer and Stammer (1939). The last mentioned men described the properties and made immunological studies on the organism, *Bacterium hemophosphoreum*. This form can be readily grown on culture media and will infect other insects when injected into the body.

Other reported luminous insects such as mole crickets (Kirby and Spence, 1817; Ludwig, 1871), may flies (Hagen, 1873; Eaton, 1880, 82) and ants (Wheeler, 1916) have probably been infected with luminous bacteria. Haneda (1939) has definitely proved the bacterial infection of a wood louse and a millipede. The luminescence of all the above animals will be described in greater detail under the groups concerned.

SYMBIOTIC LUMINOUS SPECIES

The idea that bacteria might be responsible for the light of other animals appears to have been made by Dubois (1888, 89), who found luminous bacteria in the siphon of the mollusc *Pholas* and in the luminous slime of the jelly-fish, *Pelagia*. However, he (1890) quickly withdrew the idea in favor of self-luminosity and the existence of luciferin and luciferase in *Pholas*. In 1907, Kubnt suggested that lampyrid luminescence was due to luminous bacteria, and in 1912 Osorio described luminous bacteria secreted from a gland in the belly of the fish, *Malacocephalus*. Lampyrid luminescence is certainly not bacterial in origin but the fish luminescence is symbiotic and many other fish with symbiotic luminous bacteria have been described in recent times. Osorio's discovery was largely overlooked and Molisch (1912) regarded the idea of light symbiosis as defunct.

However, Pierantoni (1914), in the case of lampyrids, and Buchner (1914), in the case of *Pyrosoma*, revived the idea and both authors have been ardent supporters of the view in numerous papers and books (see particularly Pierantoni, 1914-36; Buchner, 1918-30; Mortara, 1924; Puntoni, 1925; Meissner, 1926; Pierantoni and de Lerma, 1939). Caullery (1921) and Nuttall (1923) have reviewed the subject. The question of symbiotic luminescence will be discussed in more detail under the various groups of animals concerned, which have included fire flies, jelly-fish, ctenophores, pennatulids, earthworms, *Phyllorhoe*, *Pyrosoma*, euphausiids, squid, and fish. At this point it is sufficient to state that bacterial symbiosis is questionable among all the above groups except the squid and the fish.

There is no doubt of bacterial symbiosis in fish. The various types

of light organs resemble glands and are open to the exterior, although the bacteria are not always extruded as a secretion. The families concerned are the Macruridae, Gadidae, Monocentridae, Anomalopidae, Acropomatidae, Leiognathidae, Serranidae, and possibly the Saccopharyngidae and the Ceratioidea or deep sea angler-fishes. The two latter groups are deep-sea forms, but the others are surface fish or from medium depths. In many cases the bacteria have been isolated and grown in pure culture. True deep-sea fish of the myctophid or stomiatid type, with photophores do not appear to contain luminous bacteria.

In the case of squid there is no doubt that luminous bacteria exist in a large number of the shallow water Myopsida, which have special internal glands to harbor them. The question revolves around the definition of symbiotic. In certain species, no light can be detected in some individuals, whereas in other individuals the light and the bacteria are present. The bacteria can be easily grown on artificial culture media and Pierantoni, Zirpolo, and Meissner speak of true symbiosis. Immune reactions of the bacteria have been used in support of the view. Mortara, Skowron, and Kishitani, on the other hand, are inclined to consider the gland as a convenient nutrient medium for luminous bacteria but deny that a true symbiosis exists as the bacteria are not always present. The most controversial squid is *Heteroteuthis*, one of the Myopsida, a deep sea form which propels a luminous secretion into the sea water through the funnel. The secretion is filled with granules which might be much modified bacteria, but they cannot be cultivated and their behavior is quite different from other bacteria. The true deep sea squid belonging to the Oegopsida, do not, as far as is known, contain luminous bacteria in their photophores.

STRUCTURE

The older methods of preparing and staining bacteria for microscopic examination reveal little more than the form and the presence or absence of flagellae, capsules, and spores. Examination of living luminous bacteria by transmitted light or dark field shows the presence of granules within the cell which may be photogenic granules although proof is lacking. Electron microscopy, which has been used with 8 species including salt and fresh water forms by Johnson, Zworykin, and Warren (1943), is rather disappointing for internal structure but reveals the flagellae well and the presence of a definite wall. In *Achromobacter harveyi* there appeared to be two flagella types averaging 40 and 16 $m\mu$ in diameter, although it is possible that the larger ones

represent several of the smaller units. Both salt water and fresh water forms give evidence of cytolysis with loss of protoplasmic contents but no great swelling when placed in distilled water. The surface wall becomes cracked and often separated from a mass of coagulated protoplasm, thus confirming the conclusions which had been drawn from physiological studies of distilled water osmolysis. The improved electron microscope techniques of the present day would undoubtedly reveal more of protoplasmic structure.

The clearest picture of the bacterial cell has come from the Robinow method of fixing and staining in which the bacteria are not dried on a glass slide but are fixed with osmic acid in a thin film of agar and stained in Giemsa's blood stain. The study of Johnson and Gray (1949) has revealed some remarkable nuclear structures and peculiar bacterial shapes in the coccoid species, *Photobacterium phosphoreum*, and the rod-shaped *Achromobacter fischeri*.

In *P. phosphoreum* nuclear material appears as a dark stained central body or in two or more units resembling stages of mitotic cell division. In *A. fischeri* the long rods contain many small units of chromatin varying in shape from sphere to spheroid to dumbbell or in the form of a V or Y. These nuclear bodies are shown in Fig. 3. Very early in the growth of *A. fischeri* "large bodies," i.e., cell formations more or less spherical in shape, sometimes 7 or 8 times the diameter of the bacterial rod, appear in the cultures. Their nuclear material takes the form of a network or a clumped thread or a single mass. They are frequently connected with a filament containing a thread of chromatin. They develop by terminal or lateral outpocketing of normal cells and soon become covered by growth of rods so that their history could not be determined. Fig. 3 shows some of these large bodies. Warbasse and Johnson (1950) have shown that penicillin greatly increases their number.

When the salt is dialyzed away from a thin film of agar containing a growth of *A. fischeri* the cells cytolize and the nuclear material becomes less deeply staining, disintegrates, and finally disappears. The process can be reversed, if it has not proceeded too far. In concentrated salt up to 20% the chromatin aggregates into very distinct and dense masses.

Occasionally *A. fischeri* on a maintenance medium will form non-luminous dissociates which are not distinguishable from the luminous forms and which present the same nuclear structures. The nuclear phenomena observed in luminous bacteria have also been observed in unrelated non-luminous species.

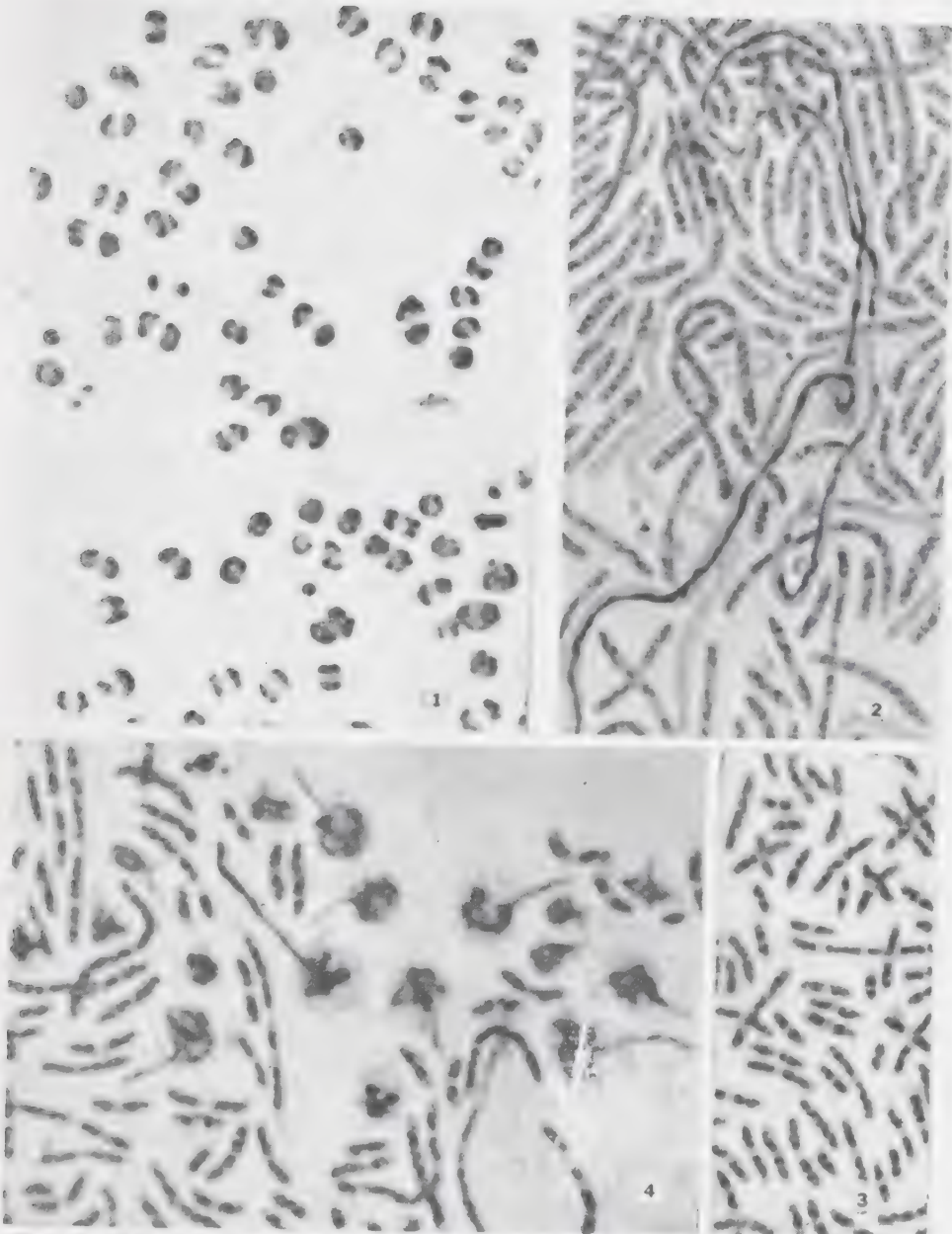


FIG. 3. Luminous bacteria stained to show nuclear material. (1) Young cells of *Photobacterium phosphoreum*. (2) *Achromobacter fischeri*, 1.75-hour culture. (3) Same, 6-hour culture. (4) Same, 4-hour culture showing "large bodies." After Johnson and Gray, in the *Journal of Bacteriology*, published by Williams and Wilkins Co.

PURPOSE OF THE LIGHT; USES TO MAN

The light emission of higher organisms may have a definite purpose — for attracting the sexes, as a lure for food or a warning signal, perhaps for illumination — but it is practically impossible to assign a function of value to luminous bacteria in the above sense. Light production must be thought of in terms of some chemical reaction, not actually necessary for the life of the bacterial cell or the bacterial economy, which happens to be chemiluminescent. The light-producing reaction is in the same category as the pigment-forming reaction of pigmented or the fluorescent compound of fluorescent bacteria. Under certain conditions production of pigment or luminescence will cease, but the life of the bacteria goes on.

Although apparently unnecessary for the bacteria themselves, light production is of value in the classroom or laboratory, as a test for small amounts of oxygen and as a check on possible cracks in bacterial filters, both uses due to Beijerinck (1889). He demonstrated that if a filter candle is cracked, some luminous bacteria will pass but sound filters keep back these forms. Luminous bacteria may also be used as an assay and toxicity test for antibiotics. Bacterial lamps have been suggested by Dubois (1900, 01) and Molisch (1904, 12) as a safe method of lighting powder magazines and a popular account given by Damon (1914). Growths of bacteria on agar were even considered as a means of marking during blackouts in Japan in World War II. Their use as a water paint was recommended by Bryson (1940). Needless to say there are other easier, more effective and more economical ways of attaining these ends.

IN VIVO REACTIONS

Most luminous bacteria grow best at relatively low temperatures and would not thrive in warm-blooded animals like birds and mammals even though no defense mechanisms were developed by the host. Luminous bacteria have not infected man, even though meat covered with them was eaten. The experiment was carried out by Tollhausen (1887), who sprinkled a meal with a luminous bouillon culture of *Bacterium phosphorescens* and ate up to 25 cc of the luminous material on three successive days without ill effects. He had previously fed the above luminous meal to a cat for three days and injected 5 cc of the bouillon culture subcutaneously into guinea pigs on three different occasions without hurting the animals. The experience of others with warm-blooded animals has been the same.

When injected into cold-blooded vertebrates, luminous bacteria do gain a foothold and will develop. Tarchanoff (1901, 02) found that

luminous bacteria obtained from the Baltic Sea and introduced by way of the dorsal lymph sacs of frogs, made the animals luminous, particularly the tongue. After three to four days the light disappeared and the frogs appeared quite normal.

In this connection it is interesting to find that, according to Kuhn (1927), the luminescence of certain bacteria is diminished or lost by passage through mice but greatly increased by passage through frogs. The strains studied were highly virulent to frogs but not to mice, guinea pigs, rats, or rabbits.

Among invertebrates the injection of luminous bacteria will result in a luminous bacterial disease which may or may not be fatal. The earliest experiments were made by Giard and Billet (1889) and Giard (1890) on various amphipod crustacea, using a bacterium which had infected sand-fleas, and Russel (1892) infected *Palaemon* with Giard's bacillus at Naples. Later Skowron (1926) infected *Amphioxus*, the marine shrimp, *Palaemon*, the squid, *Sepia*, and the tunicate, *Ciona*, with luminous bacteria from the squid *Sepiolo*. The bacteria grew particularly well in *Ciona*, making the ovary highly luminescent.

One of the earliest studies on immune reactions was by Ballner (1907), who found that serum of guinea pigs allowed to stand several days in order to rid it of bactericidal properties would agglutinate strains of luminous *Vibrio rumpel* without changing to any great extent the light emission. This result was taken by Ballner to indicate that during agglutination no harmful changes occurred in the bacteria.

These effects were reinvestigated by Ninomiya (1924), using a non-motile gram negative marine form from the Kiel Institute. He found that small amounts of active or inactive normal serum (rabbit and guinea pig) increased the light intensity, presumably due to a nutritive effect, but that inactive immune serum decreased the light due to agglutination because of a decrease in the effective surface of the bacteria through which the light was emitted. Addition of immune serum and complement still further reduced the light intensity, due to a harmful action.

Johnson (1941) obtained the same result with four different kinds of bacteria, two salt water and two fresh water species. He also determined that the salt water bacteria, cytolyzed by placing in distilled water, were agglutinated by homologous (but not by heterologous) antiserum (rabbit) in the same titer as intact living bacteria. The cell-free filtrates of these cytolyzed suspensions showed specific precipitation with homologous antiserum, indicating that antigen must come out of the bacteria on cytolysis.

This work was continued by Warren (1944) who studied eight

different species and also found the luminescence to be unaffected by agglutination. His statements are as follows: "Cross agglutination results indicate that specific agglutinogenic properties are present in *Achromobacter fischeri*, *Bacillus sepiae*, *Bacillus pierantonii* and *Photobacterium phosphoreum*. Cross reactions occur between *Achromobacter harveyi* and *Photobacterium splendidum*, between *Photobacterium splendidum* and *Bacillus sepiae*, and between *Vibrio albensis* and *Vibrio phosphorescens*. The nature of the *V. albensis* *V. phosphorescens* kinship is different from the other cross reactions. At least two major antigens are present in *V. phosphorescens*, whereas *V. albensis* contains a major and minor antigen." Agglutinin absorption tests were carried out and it was found that filtrate antigens of luminous bacteria will give rise to agglutinins which exhibit specificities characteristic of luminous cell agglutinins.

Most of the work on luminous bacterial immune reactions has not been concerned with the effect on luminescence *per se* but has been directed to differentiating between symbiotic, parasitic, and saprophytic types. The principal contributions have been made by Meissner (1926), Kishitani (1930, 32), and Majima (1931).

Meissner isolated four strains of a luminous *Vibrio Pierantonii* from the light organ of the squid, *Sepiola intermedia*, and nine strains of luminous *Coccobacillus Pierantonii* from the light organ of the squid, *Rondeletia minor*. These symbionts had been previously described by Zirpolo (1917). From the skin and muscles of non-luminous *Sepia officinalis*, six strains of saprophytic luminous *Bacillus sulla sepia* and two strains of saprophytic luminous *Vibrio sulla sepia* were isolated. Meissner found that *B. sulla sepia* shows no similarity to the symbionts but *V. sulla sepia* is culturally and morphologically similar to *V. Pierantonii* but serologically quite distinct. In general, symbionts are serologically quite distinct from ordinary luminous bacteria, as different as the typhosus and coli groups. The saprophytic luminous bacteria show no strain specificity while the symbionts show a marked strain specificity.

Meissner showed that luminous bacteria are non pathogenic for rabbit and guinea pig, but produce abscesses in *Sepia officinalis* due to a poison present in dead bacteria. Blood serum of *Sepia officinalis* was found to contain normal agglutinins for non luminous bacteria of the accessory rudimentary gland and for various luminous bacteria. These agglutinins could be increased markedly by immunization with luminous bacteria but bacteriolysis could not be demonstrated in *Sepia* immune sera. Organ extracts of *Sepiola intermedia* and *Rondeletia minor* exhibit no agglutinating, complement-fixing, precipitating, or

bacteriolytic antibodies against their own luminous bacteria. Meissner regarded this fact together with strain specificity as strong evidence for the symbiotic nature of these forms.

Kishitani (1930) found a similar strain specificity in agglutination for symbiotic *Micrococcus physiculus*, isolated from the light organ of the fish, *Physiculus japonicus* and (1932) for symbiotic *Pseudomonas euprymna*, *Micrococcus sepiola*, and *Coccobacillus loligo*, isolated from squid. Majima also was able to differentiate pathogenic *Vibrio yasakii* by immune agglutination reactions.

BACTERIOPHAGE

Attempts to obtain a phage for luminous bacteria have encountered difficulties. Epstein (1930) observed that the scum on the tanks of the aquarium at Monaco contained a *Coccobacillus* which showed a "phosphorescence inconstante" and he could detect a lytic agent (bacteriophage) in the tanks which remained there for a long time. In 1930 also, Sonnenschein (1931) isolated a *Vibrio albensis* from a fish of the Elbe River which grew and luminesced well on an ox gall medium, but he was unable to find a phage for the organism in Elbe water or Hamburg water or to obtain one from fish or feces of various animals. Later Sonnenschein (1932) did succeed in adapting a cholera vibrio phage to *V. albensis*. Adaptation was brought about by starting a culture of cholera vibrio with cholera phage and then adding some *V. albensis*. The filtrate from this culture was then used with a new culture of cholera vibrio and more *V. albensis* added. After five passages the phage would attack the luminous *V. albensis* also and could be enhanced by further passage. The new phage caused complete lysis and disappearance of light, but the light would reappear during secondary growth. The phage was also active on dark mutants obtained from old colonies of *V. albensis* and secondary growth after such action gave only dark colonies, but light and dark colonies developed after the action of phage on luminous strains of *V. albensis*. No mucous forms appeared as a result of phage action.

Isolation of a luminous bacteria bacteriophage system has also been made by M. B. Baylor.* The bacteria were similar to *Achromobacter harveyi*, obtained from dead fish at Bermuda, and the phage found in a brackish pool at the same place. Electron microscope studies show the phage to resemble the coli phages in size and shape. The absorption capacity is about 25 phage per bacterium, and the burst size around 200 phage per bacterium. A remarkable aspect of the behavior, observed with living cells on a nutrient medium under a light micro-

* Private communication of observations to be published in 1951.

scope, is the growth and division at 23°C. of infected bacteria, which would lyse if kept at 28°C. It was not possible to separate the growth and lytic processes in liquid culture.

If all the bacteria lyse after the constant period (about 54 minutes at 28°C.), luminescence intensity remains constant until lysis occurs, when the light disappears. If, however, conditions are such that only part of the cells lyse, a drop in luminescence is correlated with the partial lysis followed by a rise as the remaining infected cells grow and divide. The luminescence picture is complicated by the ability of the cells either to lyse or to grow in the presence of the phage.

CULTURE MEDIA AND METABOLISM

Luminous bacteria will grow, if the temperature is relatively low, on almost any medium which is not too acid or alkaline, as might be expected from their wide distribution in nature. They usually thrive on fish, meat, eggs, and sausage and will live on cooked potatoes, provided the potatoes have come in contact with flesh or fish juices.

Lehmann (1889) and Beijerinck (1889) used milk as a culture medium. The author (1920) has observed some growth, although not too abundant, on urine and it is possible that some reported cases of human luminescence have been due to growth on sweaty exudations of the skin. Sommenschein (1931) found that a luminous *Vibrio* grew well on ox gall, but Ziker (1912) was unable to grow *Bacterium phosphoreum* or *Pseudomonas lucifera* on wort, hops, or wort and beef extract, with or without gelatin, even if 3% salt was added.

An excellent medium for most luminous bacteria is easily made up with 1.5% agar, 1% beef extract, 2% peptone, and 1% glycerine* to which some powdered CaCO_3 has been added to maintain the pH at a slightly alkaline value. In the case of sea water species the materials must be dissolved in 3% NaCl or sea water, whereas the fresh water forms do well with 0.5% NaCl. For experimentation, the 12 to 24 hours' growth on agar in petri dishes at room temperature (20°C.) may be removed with a camel's-hair brush and suspended in a well-buffered solution known as PN, which is made by mixing equal volumes of $m/2$ NaCl and $m/4$ phosphate buffer at pH 7.3 (8 parts $m/4$ NaH_2PO_4 and 2 parts $m/4$ KH_2PO_4). The suspension in PN, to which 1% glucose may be added, glows with a bright constant light intensity for a long period, provided sufficient oxygen is supplied by aeration. The light of a suspension in sea water continually decreases, due to lack of nutrient and insufficient buffering.

Studies on the growth requirements of luminous bacteria have been

* According to Farghaly (1950), 0.3% glycerine is a better concentration.

too numerous to report in detail. One of the earliest was by Beijerinck (1889, 91) who introduced the method of "auxanographie" to determine what the effect of substances on light and growth might be. He grew the bacteria on a solid medium with insufficient nutrients so that growth and luminescence soon ceased and then added a drop of the substance to be tested, which diffused in all directions. Luminescence reappeared if the material was a "light nutrient" and growth began if a "growth nutrient." Beijerinck's table (1891, p. 411, reproduced by Molisch, 1904, 12) distinguished those materials which increased light and growth such as glucose, those having no effect, such as lactose, and those with a poisonous action like vanillin. It is interesting to note that he found the chemiluminescent substances, lophin or amarine, to have no effect on the luminescence. The auxanogram has been used by Johnson, Carver, and Harryman (1942) to study luminescence inhibition and luminescence stimulation by heavy metals and various drugs and antibiotics.

The utilization of nutrient in the culture medium frequently explains the unequal luminosity of bacterial colonies growing on an agar plate. Such effects have been beautifully demonstrated by Cruickshank (1934) from photographs by their own light of colonies symmetrically inoculated on agar or gelatin. Wherever the diffusion of nutrient to the growing colony is insufficient, dimming or extinction of light will result.

Carbohydrates and Polyhydric Alcohols. Beijerinck found that peptone alone would not adequately support growth or luminescence but that a carbohydrate was also necessary. This statement regarding carbohydrate must be modified, as many other carbon sources like organic acids or glycerine and polyhydric alcohols can be substituted. If bacteria are thoroughly washed to remove all nutrient, there is still some luminescence and a low oxygen consumption, referred to as the endogenous respiration. The exogenous respiration is the additional oxygen consumption observed by Beijerinck and many other workers (Gerretsen, 1915, 20; Taylor, 1932, 34; Johnson, 1935, 39; van Schouwenburg, 1938) when carbon food sources are added.

Beijerinck observed that *Photobacterium phosphorescens* luminesced with maltose but *Ph. pflügeri* did not. However, if traces of maltase were placed on the dark petri plates inoculated with *Ph. pflügeri*, bright colonies appeared which he explained as due to hydrolysis of the maltose, illustrating another use of the anauxogram to test for activity of enzymes. It is possible that adaptive enzyme formation may have occurred here.

A very large number of recent papers have dealt with the utiliza-

tion of different sugars, by Chodat and de Coulon (1916), de Coulon (1916), Majima (1931), Fuhrmann (1932), and especially Johnson (1935-39). Yasaki and Nomura (1947) have studied glycerine as a nutrient.

In general it may be said that different species of luminous bacteria can utilize different sugars, just as has been found for other non-luminous types of bacteria. Majima (1931) tested 56 strains of luminous bacteria, including saprophytic, symbiotic, and pathogenic forms, all of which fermented dextrose, levulose, and mannose but not adonite, inosite, nutrose, raffinose, and sorbite. The saprophytic and symbiotic forms fermented xylose, but the pathogenic forms (*Microspira phosphoreum* of Yasaki, from fresh water shrimp) did not.

The most comprehensive study of carbohydrates, carbohydrate alcohols, and allied substances has been made by Johnson (1935-39), who used the washed bacteria technique. Brightly luminescent bacteria of two species, the fresh water *Vibrio phosphorescens* and the marine *Achromobacter fischeri* were brushed from petri dishes, thoroughly washed and suspended in phosphate buffer at pH 7.3 together with the added nutrient. The oxygen consumption of the washed bacteria and that of the washed bacteria + nutrient was then determined as a measure of utilization. The very low endogenous metabolism of washed bacteria immediately increased if the carbohydrate was utilized. Of 28 substrates, only dextrose, fructose, sucrose, trehalose, melicitose, mannose, glycerine, maltose, alpha-galactose, raffinose and sorbit were oxidized by one or the other organism and the more rapid the oxidation, the brighter the luminescence. Among monosaccharides and their alcohols, only those with three or six carbon atoms were oxidized. The marine species could oxidize only reducing compounds, with the exception of glycerol and melicitose.

Addition of several carbohydrates simultaneously to the medium was studied and a competitive action demonstrated in the case of some carbohydrates which were not oxidized, provided they were similar in molecular configuration to those which could be oxidized, but the competitive inhibition was not always observed even though structural configuration was marked. One of the most interesting inhibitors was alpha methylglucoside which prevented the oxidation of fructose, mannose, galactose, and glucose, depending on its concentration. The inhibition of oxidation also inhibited the luminescence.

In a series of papers, Johnson (1937, 38) and Johnson and Anderson (1938) have analyzed the effects of glucosides and other related substances on oxidation of carbohydrates. There may be either com-

plete inhibition or retardation of oxidation, depending on the glucoside or, depending on the sugar, one glucoside may increase or decrease the oxidation. Luminescence was not markedly increased by any compound but was usually decreased, particularly by kojic acid, which acted without affecting the oxygen consumption of washed cells.

From low concentrations of glucoside there is a sudden "escape," after a period of time, with complete recovery of respiration and luminescence. The reduction of methylene blue under the above conditions was also studied and many detailed experiments carried out which must be obtained from the original communications.

The study of sugar metabolism in relation to luminescence led Johnson (1938) to the view that well-washed cells of luminous bacteria suspended in phosphate buffer contain a definite amount of luciferin which, in the presence of a carbohydrate substrate, such as glucose or fructose, is irreversibly oxidized during the luminescent reaction, and is not renewed. Quantitative data showed that, although the rate of oxygen consumption remains high and nearly uniform for several hours, the intensity of luminescence may, in the same interval, decrease by more than 90%. In addition qualitative observations indicated that the decrease in luminescence with time was caused neither by the exhaustion of carbohydrate substrate, nor by the accumulation of harmful metabolites. This view was tested by Johnson (1939) in an experiment in which known amounts of glucose were added to a washed cell suspension after successively longer periods of aeration. Although the resulting increase in light intensity appears more slowly and the maximum intensity attained is less, the total luminescence over a period of some hours is approximately the same. Johnson concluded that an "irreversible luminescent oxidation of luciferin by luciferase in bacteria follows a reduction of an oxidized form of luciferin (different from that resulting from luminescent oxidation) by dehydrogenase activity. The general form of all the curves for luminescence intensity in relation to time after adding glucose may be described by differential equations on the basis of these two reactions." These studies lead to the question of the relation of luminescence to cell respiration, which will be discussed in a later section.

Farghaly (1950) found that among ten sugars tested with *Achromobacter fischeri* only glucose could be utilized. Lactose, maltose, sucrose, fructose, galactose, D-sorbose, D-xylose, D-arabinose, and L-arabinose were not assimilated but no attempts were made to adapt the organism to these compounds.

Organic Acids. Beijerinck classed a number of organic acids as light promoting substances while others had no such action, but lack

of knowledge of the pH under the conditions of study has rendered his work difficult to interpret. One of the first workers to include the acids of intermediary metabolism in the culture medium were Berthelot and Amoureux (1924). They obtained good growth and especially bright luminescence of bacteria in a medium containing asparagine, peptone, choline HCl, salt and phosphate, when 4 g per liter of sodium pyruvate was added. Johnson, van Schouwenburg and van der Burg (1939) have pointed out that pyruvate added under anaerobic conditions can augment the flash when oxygen is readmitted, while succinate, not metabolized anaerobically, cannot. However, according to Claren (1938), succinate is formed anaerobically by such forms as *Mikrococcus cyanophos* when fumarate is added, the latter acting as hydrogen acceptor. A few experiments have been made with formic, pyruvic, and fumaric acids under anaerobic conditions by Doudoroff (1942) and under aerobic conditions by Giese (1943).

Another investigation of the organic acids as sole source of carbon in luminous bacterial metabolism has been carried out by Farghaly (1950), using *Achromobacter fischeri*. He found that this organism was unable to utilize the sodium salts of pyruvic, lactic, malic, fumaric, succinic, citric, acetic or alpha-ketoglutaric acids.

Nitrogen Sources. Apparently nitrates and nitrites cannot be used as sources of nitrogen by luminous bacteria and the usual nitrogen supply comes from protein or its degradation products. One experiment suggests possible utilization of atmospheric nitrogen, but must be further investigated before acceptance. Mudrak (1933) observed growth and luminescence after five successive inoculations on a medium composed solely of 0.02% CaCl_2 , MgSO_4 , and K_2HPO_4 , a trace of FeSO_4 , 2% NaCl and 0.5% glycerine dissolved in conductivity water and protected from ammonia of the air. He was prevented by lack of time from continuing the study of this remarkable result, which was pronounced in one particular strain isolated from fish.

Although Beijerinck stated that ammonium salts of organic acids were not assimilated by his luminous bacteria, later work has indicated that ammonium salts can serve as sole source of nitrogen. Farghaly (1950) tested ammonium chloride, nitrate, sulfate, tartrate, citrate, and $\text{NH}_4\text{H}_2\text{PO}_4$, finding no appreciable difference in luminescence intensity of *Achromobacter fischeri* after 48 hours' growth, although the light diminished more rapidly with ammonium nitrate, chloride, and sulfate. NaNO_2 and NaNO_3 and some other nitrogen sources would not support growth or luminescence.

Beijerinck found that in combination with peptone, asparagine was particularly active in stimulating luminescence, and McKenny (1902)

also claimed that certain amides stimulated growth. Some amino acids were studied by Beijerinck by the auxanographic method, but detailed knowledge of the amino acids necessary for luminescence has only come after pure samples were commercially available. Gerretsen (1920) made a few experiments with amino acids and Mudrak (1933) showed very clearly that asparagine, aspartic acid, and glycocoll could take the place of peptone and the cultures would luminesce well, if buffers were present. Tyrosine and urea were inhibitory. Bukatsch (1936) found that 3% NaCl with small amounts of other salts and 1% glycerine to which had been added 0.01 to 0.05% glutamic acid, serine, leucine, or alanine gave a very bright long lasting light. Arginine and valine gave a weaker light and tyrosine and creatine appeared to be inhibitory.

The work of Doudoroff (1942) will illustrate the complexity of the subject. He studied a number of strains, using as a basal medium, distilled water containing *m* 30 KH_2PO_4 - Na_2HPO_4 phosphate buffer mixture at pH 7.0; 3% NaCl; 0.03% NH_4Cl ; 0.03% MgSO_4 ; 0.001% FeCl_3 ; 0.001% CaCl_2 ; and 0.1–0.3% of the organic carbon source by weight. No attempt was made to control in detail the mineral nutrition of the organisms, and all essential trace elements were assumed to be present in the reagents used. The carbon source was usually glycerine, glucose, lactate, succinate, or fumarate.

Different bacteria reacted differently and even the previous history of the bacteria determined nutrient requirements. Doudoroff wrote: "Photobacterium fischeri, Ph. splendidum, Ph. seipiae and Achromobacter harveyi were found to be capable of developing in inorganic media with simple organic compounds as the sole carbon source, while most strains of Photobacterium phosphoreum did not grow unless methionine was added as an accessory factor. In experiments with one strain of Ph. phosphoreum, no compound or combination of compounds tried could replace methionine. Among the ten other strains tested, one was found for which no accessory factor whatsoever was essential, although methionine had a growth-promoting effect. Cultures of another strain studied could be 'weaned' from methionine to accept homocystine as substitute, or even to develop in the absence of any accessory factor. The complementary action of leucine and methionine, the 'detoxification' of media to which certain inhibitory compounds were added by the addition of other compounds, and the stimulating effect of a variety of substances on the initiation of growth in certain media were observed in the studies with Ph. phosphoreum."

Norleucine is one of the compounds which may inhibit growth of *A. fischeri* and its "detoxification" or reversal has been studied by

Daniel (1950). He found that homocysteine, cystathionine, and homoserine reverse norleucine inhibition of growth in a manner suggesting that they are precursors to methionine. Contrary to the results obtained with other organisms methionine appears to be a product of the inhibited reaction while valine, leucine, and isoleucine, apparently function in the biosynthesis of methionine or one of its precursors since they reverse norleucine inhibition in a competitive manner. The results suggest that methionine and valine, leucine, and isoleucine are related to one another through a transamination reaction which is sensitive to norleucine.

The importance of methionine has been again pointed out by Farghaly (1950), who found the luminescence of *A. fischeri* to lag behind growth in early multiplication of this form, apparently due to a competition between growth and luminescent systems for methionine. When added to a basal medium the lag is greatly shortened and the luminescence intensity increases. Lysine and some other amino acids also increase the light intensity but do not reduce the lag. A detailed study of other amino acids will be found in Farghaly's paper and discussion of the nutrient activity of both amino acids and vitamins in a communication of Okochi (1949).

GROWTH CURVES

The growth of luminous bacteria, a strain allied to *Achromobacter fischeri* isolated from a surface fish at Bermuda, has been studied quantitatively by Baylor (1949). Inoculations were made in a tube of 2% nutrient broth containing 3 parts beef extract and 5 parts peptone, enriched with 1% glycerine. Air was continually bubbled through the broth during the period of growth. Light intensity was automatically recorded with a photomultiplier tube and density of the developing suspension by a turbidometer technique, using a beam of red light traversing the tube. The turbidity measurements were calibrated by plate counts of bacteria.

As in the case of other bacteria, five cycles of growth can be recognized. (1) the initial stationary phase, sometimes referred to as the latent period, occurring immediately after inoculation, during which the number of bacteria remains virtually constant; (2) the lag phase, during which the rate of increase in cell count is progressively accelerated to the maximum constant rate which is (3) the logarithmic growth phase, throughout which the rate of increase of cell count remains the same up to (4) the phase of negative acceleration of growth, when the rate of increase in cell count is decelerated, to taper off the growth to (5) the plateau stage, or maximum stationary phase.

during which there is no increase in cell count. The generation time, i.e., the time for the cell mass to double is about 60 minutes at a temperature of 28°C.

Luminescence intensity exhibits phases similar to the growth cycles. When total light produced and total cell count are compared, the curves are very similar, except that during the initial stationary phase of growth the light intensity is more variable than growth rate, and after the peak of light intensity has been reached, the luminescence decreases immediately, without a plateau, through a series of lesser peaks. This period constitutes the phase of negative acceleration of luminescence. It declines to a value about one-tenth that of the peak

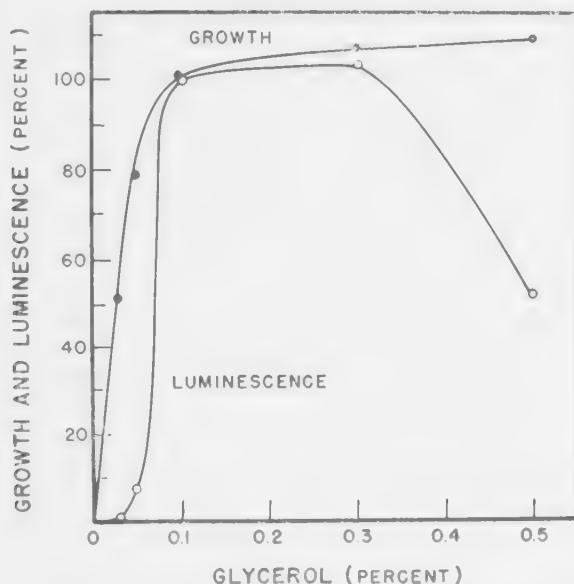


FIG. 4. The relationship between growth, luminescence, and glycerol concentration for *A. fischeri*. The results are expressed on a percentage basis considering the highest value of luminescence obtained and the corresponding amount of growth as 100. The incubation period was 66 hours at 23°C. After Farghaly.

and then remains nearly constant, corresponding to the plateau stage of growth. Obaton (1938) had previously noted that the luminescence decreased in the course of successive divisions.

The light intensity per bacterium was also studied and found to be greatest at the peak of luminescence intensity, indicating that this maximum is not entirely due to number of bacteria but to increased light emission of each bacterium as well.

It must not be forgotten that growth curves and the relation of luminescence to growth depend greatly on the culture medium. The growth curves of Farghaly (1950) indicate that on an "improved"

basal medium luminescence of *Achromobacter fischeri* does not develop in appreciable amounts until 30 to 40% of maximal growth has taken place. As previously indicated, this lag can be greatly shortened by addition of methionine. Glycerol was found to be inhibitory of luminescence in high concentrations although the effect on growth was not marked. These relations of growth and luminescence in presence of glycerol and methionine are shown in Figs. 4 and 5.

MUTANTS

Most workers on luminous bacteria have noticed their pleomorphic character and a frequent tendency for the luminescence to become less

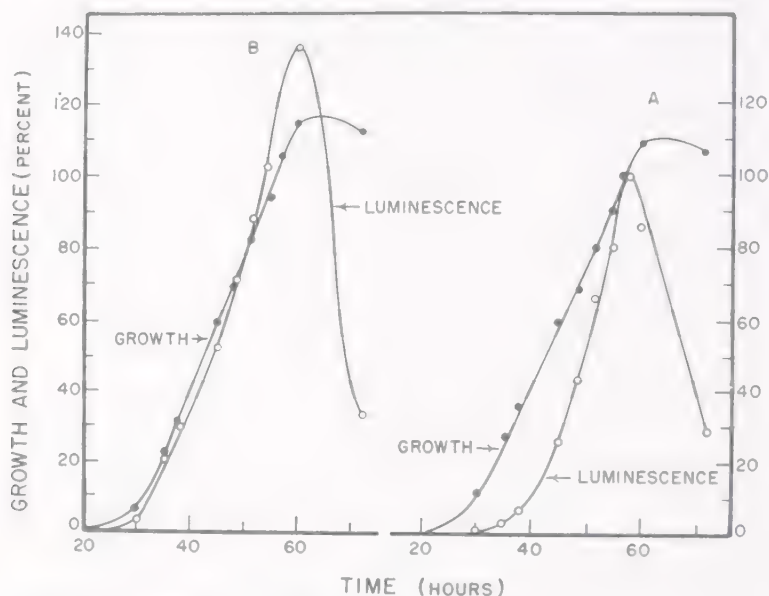


FIG. 5. Rate of growth and light production of *A. fischeri* on the basal medium (A) and on the basal medium plus 30 mg methionine per 10 ml (B). The results are expressed on a percentage basis considering the highest value of luminescence and the corresponding amount of growth as 100. After Farghaly.

bright when the bacteria are continually transferred on the same artificial culture medium. This change in light intensity depends on the species of bacterium. With certain species dark mutants arise spontaneously or may be produced by appropriate treatment. In order that a true dim or dark mutant may arise, it is necessary that the strain retain its new character over a considerable number of transfers to new media. Some luminous bacteria will grow in absence of oxygen or in presence of certain chemicals without producing light, but light immediately reappears when transferred to normal conditions and they cannot be considered mutants.

As early as 1889 Beijerinck noticed that his old cultures of *Photobacterium indicum* or *Ph. luminosum* would sometimes emit only a weak light or no light when inoculated on a new medium. Other characteristics might also change such as ability to liquefy gelatin. An extended study was later made by Beijerinck (1912) in a long general paper entitled, "Mutation bei Microben." In dealing with luminous bacteria he stated that he had transferred fresh colonies of *Bacillus indicus*¹⁰ for twenty-five years without noticing any change in light intensity, but when grown at higher temperatures (25-30°) strains called "semiobscurus" and "obscurus" immediately appeared. They were not always completely dark, but nearly so, and could be transferred as dim mutants from one tube to another, but occasionally more brightly lighting colonies would arise among them. He also obtained strains small in size called "parvus."

Beijerinck (1915) continued his studies with the highly motile *Photobacter splendidum*, whose general characteristics are similar to those of the cholera vibrio. It has an optimum temperature for luminescence at 23-25° and for growth 29-30°. The results were entirely confirmatory. As in the case of *Bacillus indicus* growth (at 30°) above the optimum luminescence temperature gave rise to dim or dark mutants which bred true when inoculated on other media. The mutation did not occur in one jump but in steps and the intermediate stages were regarded as submutants which had great hereditary stability. Usually there was a very weak light in the mutants which could be slowly increased in all individuals by proper nourishment. Occasionally the dark mutants gave rise to single atavistic strains which were brightly luminescent and had the characteristics of the submutants rather than of the original stock. Old dark mutants were entirely dark and remained so for years. They were more stable than the original stock and were hard to distinguish from ordinary salt water *Vibrios*.

Beijerinck spoke of the light as connected with "photoplasma," which he (1917) later regarded as made up of enzymes or hereditary units, and took the existence of submutants to indicate that the photoplasma as an hereditary unit could be split. He also pointed out that the light function was like virulence since there was a great similarity in the behavior of luminescent and virulent strains of bacteria. Dubois (1919) also noticed that *Photobacterium sarcophilum* became pleomorphic when grown with lecithin under anaerobic conditions and that the photogenic capacity might be lost without change of form.

¹⁰ In the 1915 paper Beijerinck speaks of *Bacillus phosphorescens* = *Photobacter indicum*.

Doudoroff (1938) endeavored to determine the constituent responsible for the loss of luminescence. He isolated from fish a number of luminous bacteria which appeared to be similar to *Achromobacter phosphoreum*. When grown on yeast autolysate agar with 1% glycerine and 3% NaCl many of the strains produced dark (obscurus) and dim (semiobscurus) dissociates and occasionally a brighter variety. With some of the dim strains but not with all, the addition of lactoflavin (0.02 γ per cc) would effect return to normal brightness. Presumably the dim strains were unable to synthesize lactoflavin from other substances in the medium. The respiration (O_2 uptake) was the same within the limit of error in bright and dim strains and also in dim strains artificially brightened by addition of lactoflavin, leading Doudoroff to conclude that flavin or a derivative was connected with one of the enzymes involved in light production by the bacteria.

Giese (1943) has observed a bright variant (Y) of *Achromobacter fischeri* (W) which developed a yellow pigment and a brilliant long-lasting light. The liquid cultures of *A. fischeri* tend to become acid and the yellow variant appeared to have adapted itself to the acid conditions. He found that the increase of respiration on adding acids like succinic, fumaric, malic, and pyruvic was greater in the yellow strain, and glycerol was utilized more effectively under acid conditions. The Y strain appeared quickly, but dim mutants reverting to the original strain (W) occurred in old cultures.

It might be expected that radiation would induce mutation and Gerretsen (1915, 20) attempted to obtain dark mutants by treatment with ultraviolet light, but found that all colonies which survived the ultraviolet treatment would luminesce. However, the recent work of McElroy and Farghaly (1948) on *Achromobacter fischeri* indicates that mutations which affect both growth and luminescence can be obtained by exposure to ultraviolet light of 2537 Å wavelength. In this work the technique of Beadle was used—culture tests of the ultraviolet treated bacteria on an adequate medium and on a minimal medium to which was added various factors allowing either growth or growth and luminescence. By this procedure it might be possible to distinguish between (1) those strains which fail to grow on the minimal medium but grow and luminesce normally when the necessary growth factor is added to the minimal medium; (2) those which grow normally on the minimal but luminesce only on the complete medium, indicating a specific block in the light system; and (3) those which fail to grow on the minimal and subsequently show a competition between light and growth systems for the added components, indicating a block in a reaction common to both luminescence and growth.

The bacterium was *Achromobacter fischeri* and the basal medium that of Douderoff (1942), modified in the following manner: NaCl, 30 g; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 4.5 g; KH_2PO_4 , 0.7 g; NH_4NO_3 , 1 g; MgSO_4 , 0.1 g; glycerol, 10 ml; trace elements, 0.05 ml; L(+)-histidine, 10 mg; DL-threonine, 10 mg; and H_2O , 1 liter. The pH was adjusted to 7.3. A liter of the trace element solution contained the following: CaCl_2 , 2.7 g; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.96 g; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 36 mg; and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 39 mg. With the L-histidine and DL-threonine the luminescence was much brighter and the original *A. fischeri* strain would grow on this medium at 23°C.

McElroy and Farghaly analyzed the growth and luminescence requirements of five different ultraviolet induced mutants of *A. fischeri* that would not grow or luminesce on the minimal medium unless an additional factor was added in sufficient concentration. Two mutants required arginine, one required aspartic acid, one glutamic acid or proline and one guanylic acid. The behavior was as follows:

"In a strain which requires arginine for growth, the luminescence fails to develop in growing cultures until the concentration of the arginine is high enough to give 30% of the normal growth. With higher concentrations of arginine, the luminescent system develops rapidly and finally reaches the wild type level of intensity at a concentration which just gives the maximum growth. In an aspartic acid mutant luminescence develops only after the maximum growth has been attained. After the growth requirements have been satisfied, the luminescent system develops rapidly with increasing concentrations of DL-aspartic acid, finally reaching 90% of the wild type level of intensity. Supplements of a mixture of methionine, leucine, isoleucine, and arginine will prevent this lag in the development of the luminescent system. In a mutant requiring guanylic acid, there is a lag in both growth and luminescence until a critical concentration is reached, beyond which point both growth and luminescence increase rapidly and attain their maximum value within a narrow concentration range. Guanine fails to support growth and guanosine gives, at the optimum concentration, only 10% of the normal growth and luminescence. In one mutant either glutamic acid, glutamine, or proline could support growth but only 20-30% of the normal density could be obtained with these compounds. A mixture of glutamine and glutamic acid increased growth to 70% of the normal. Luminescence fails to develop, irrespective of the concentrations of glutamic acid, glutamine or proline."

Actually these growth requirements vary with the temperature. McElroy and Farghaly found that the Doudoroff basal or minimal

medium failed to support growth of *A. fischeri* at temperatures above 26–27° but would do so if hydrolyzed casein was added. Anderson (1948) analyzed the components in casein which allowed growth at the higher temperature and found that adding aspartic acid and arginine or mixtures of methionine, glutamic acid, and serine to the basic solution would allow a better growth and luminescence at 29° than would the addition of casein.

The rate of induction of mutants by ultraviolet light is very low. It was observed by Miller, Farghaly, and McElroy (1949) that a low temperature (8°C) incubation technique would increase the mutation rate over 20 times. This growth at low temperatures holds for ultraviolet induced and also for nitrogen mustard (bis-beta-chloroethyl-methylamine) induced mutants of *A. fischeri*. Under nutritional conditions just sufficient to support the normal wild type strain at the low temperature, there is a selective advantage favoring strains which have a more exacting growth requirement. This selection appears to be due to a loss of essential nutrients from the wild type cells.

LUCIFERIN-LUCIFERASE REACTION

The early workers recognized that luminous bacteria never secreted a luminous material which remained in the filtrate when a suspension was filtered. Since luminous bacteria can be easily grown in large quantity at any time of year, they might themselves serve as a convenient source of photogens, but unfortunately all attempts to extract luciferin or luciferase from bacteria have failed. Although Dubois worked with bacteria in the last century, he made no mention of testing for luciferin or luciferase. Gerretsen (1915, 20) has reported negative results with *Photobacterium phosphorescens* but a weak positive reaction with *Ph. javanense*. The bacterial mass was ground with very fine sand for $\frac{3}{4}$ hour to prepare the luciferase and the bacteria heated to 65° for 2 minutes to prepare the luciferin. It has not been possible to confirm this experiment, and all the results of Harvey (1916, 26),¹¹ Korr (1935), and van der Kerk (1942) have been negative. Since many enzymes are difficult to extract from bacteria, existing in an "endoenzyme" condition, we may presume that the difficulty arises in extraction of luciferase.

The author (1915) found that luminous bacteria could be dried over CaCl_2 on strips of filter paper and would luminesce if moistened

¹¹The author (1916) obtained light on mixing "bacterial luciferin" with "fire-fly luciferase" but the effect is probably due to adenosinetriphosphate in the bacterial extract.

with oxygen-containing water but not if the water was oxygen free. A later admission of oxygen did not result in luminescence, so that anaerobic destructive processes must have removed the photogens. The dried bacteria could be extracted with ether (cold or hot), chloroform, (cold or hot), cold benzol, toluol, petroleum ether and CCl_4 and still luminesce when moistened but no luminescence occurred if first extracted with hot absolute alcohol or cold 90% alcohol or cold acetone. After the dried bacteria had been thoroughly ground with sand and moistened no luminescence occurred.

Later (1926) the author tried every conceivable method of extracting possible photogenic substances from bacteria. One procedure for preparation of luciferin—cytolysis in absence of oxygen at various high temperatures, which might destroy active autolytic or other types of enzyme systems—was applied without success. It was also found that bacterial cells that have been cytolysed in absence of oxygen do not luminesce when air is admitted. Many unpublished procedures were also tested. A material like luciferin that would luminesce when mixed with extracts of the bacteria or with *Cypridina* luciferase could not be extracted with methyl alcohol in a hydrogen atmosphere. Bacteria frozen at liquid air temperatures and the ice removed *in vacuo*¹² luminesce momentarily when moistened and readily grow on agar plates, but the dark water extract does not luminesce with bacterial luciferin prepared in various ways or with *Cypridina* luciferin. Acetone-treated bacteria also exhibit no luminescent activity which might be attributed to bacterial luciferase.

Korr (1935) continued the attempts, using, in addition to osmotic and fat-solvent cytolysis, new methods of disintegration such as supersonic vibrations on three species of bacteria, *Vibrio phosphorescens*, *Achromobacter fischeri*, and an unidentified marine form. He also found that whenever the bacterial structure was materially altered, even in absence of oxygen, no luciferin or luciferase could be demonstrated. The respiration and reducing activity were likewise profoundly altered. Van der Kerk (1942) also tried "exhaustive" bacteria and liquid air treatment to obtain photogenic material with negative results.

Despite the inability to demonstrate these substances, all recent writers have assumed that luciferin and luciferase exist in bacteria, and in explanation of light emission postulate properties more or less similar to those of *Cypridina* luciferin and luciferase. Nakamura (1942) in a Japanese paper has referred to a coluciferase in luminous bacteria.

¹² Experiments with Dr. R. Ballentine.

ENZYMES

In addition to the presence of an enzyme acting like a luciferase, there are also present in luminous bacteria, catalase, cytochrome oxidase, various types of dehydrogenase, autolytic proteases and many others, not specifically investigated.

Catalase. Although Nakamura (1939) reported no catalase in *Micrococcus phosphoreus*, a facultative anaerobe isolated from squid, Gerretsen (1920) and all other workers have found catalase to be present. Nakamura supposed that luciferin reacted with the H_2O_2 formed by oxidative processes during light emission in luminous bacteria, thus taking the place of catalase in non-luminous forms, but this idea is undoubtedly incorrect. Van Schouwenburg (1940) determined the catalase content quantitatively in seven different species, including *Micrococcus splendidus*, and two forms from squid, *Bacillus Pierantonii* and *B. sulla sepia*. The results were expressed as the reaction velocity constant for decomposition of H_2O_2 divided by the number of bacteria in the suspension. Values varied from 1.08×10^{-4} for *Photobacterium fischeri* to 0.12×10^{-4} for *Bacillus sulla sepia*, similar to values for non-luminous bacteria. In one experiment with water-cytolyzed *Ph. fischeri*, the value was fifty times higher than with the living bacteria.

Johnson and van Schouwenburg (1939) have used luminous bacteria to demonstrate that H_2O_2 can be decomposed by catalase in complete absence of oxygen.¹³ For the experiment dilute pure H_2O_2 was placed in one vessel A, luminous bacterial suspensions in a second vessel B and additional luminous bacterial suspensions in a third vessel C. Pure hydrogen passed through the chain drove out all oxygen, and the bacteria became dark, but when vessel A was mixed with B in an atmosphere of hydrogen, the bacteria in B gave a flash of light and slightly later those in C luminesced due to liberation of oxygen from H_2O_2 in B.

Cytochrome Oxidase. No special study of this enzyme has been made, but it was observed by the author¹⁴ in 1931 that the absorption bands of the various cytochromes could be seen in rather thick suspensions of *Bacterium phosphorescens* under anaerobic conditions and that they disappeared when oxygen was readmitted. Yasaki and Kanbe (1946) in a Japanese paper have also studied cytochrome in luminous

¹³ This finding is contrary to that of Keilin and Hartree (*Proc. Roy. Soc.*, **B124**, 397, 1938) whose results were later (Keilin and Hartree, *Nature*, **152**, 626, 1943) traced to inhibition of catalase by nitrogen oxides in the nitrogen used for anoxic conditions.

¹⁴ Unpublished work in collaboration with S. Nesbitt.

bacteria. The existence of iron enzymes of the Warburg hemin type is made certain by the various experiments on cyanide and carbon monoxide inhibited respiration described in later sections.

Tyrosinase. This enzyme is presumably responsible for a brown coloration which appears in culture media to which tyrosine has been added during growth of *Bacterium phosphorescens* according to Lehmann and Sano (1908).

Flavin Enzymes. These undoubtedly occur in luminous bacteria and are probably responsible for the cyanide-insensitive respiration found by Eymers and van Schouwenburg (1937) and van Schouwenburg (1938). Rottier (1942) and van der Kerk (1943) isolated riboflavin tetraacetate from *Photobacterium phosphoreum*.

Hydrogenlyase and Hydrogenase. These enzymes are concerned, respectively, with the liberation or utilization of hydrogen and are known to be present in luminous bacteria. Clarens (1938) showed that *Micrococcus cyanophos* could use hydrogen in presence of fumaric acid. Yamagata and Nakamura (1937) found hydrogenase in *Micrococcus phosphoreus* and Nakamura (1940) has stated that luminescence occurs in hydrogen in presence of fumarate. Beijerinck (1889) found hydrogen to be produced by *Photobacterium phosphorescens* and Nakamura (1940) has also measured the hydrogen produced when *Micrococcus phosphoreus* grows under anaerobic conditions with glucose, formate, glycerine, or glycerophosphate in the culture medium. This hydrogen can be transferred to various acceptors in presence of hydrogenases.

Dehydrogenases. These enzymes have not been isolated from luminous bacteria but are undoubtedly present, in view of the reducing properties described later.

Hydrolyzing Enzymes. Again, no special studies of protein, fat, or carbohydrate hydrolysis by luminous bacteria have been made, except for the observation that certain species can utilize di- or trisaccharides or liquefy gelatin. The existence of autolytic protein-splitting enzymes has been deduced from the behavior of bacteria under anaerobic conditions, particularly in connection with cyanide effects and with the flash.

RELATION TO OXYGEN

Diffusion of oxygen into a liquid medium not disturbed by convection currents is slow. Because of this fact and the relatively rapid utilization of oxygen by luminous bacteria in respiration and luminescence, masses of these organisms rapidly consume all available oxygen and become dark except for a surface film in direct contact with

air. Because of the rapid utilization of oxygen, early workers had no difficulty in proving that the light of dead fish or flesh disappeared in neutral gases like N_2 or H_2 , even though these gases may have contained traces of air. After the bacterial nature of luminous flesh was established, practically all workers on growth in pure culture emphasize the necessity of oxygen for light production. Frankel (1889) found abundant growth in pure CO_2 , but no light production and Faraghalý (1950) has recently reported that on a basal medium no growth of *Achromobacter fischeri* occurs in absence of CO_2 .

Some luminous bacteria are facultative anaerobes. They will grow in absence of oxygen even though they do not luminesce. According to the observations of Beijerinck (1889) and Lehmann (1889) *Photobacterium phosphorescens* is a facultative anaerobe while *Ph. luminosum* and *Ph. indicum* are strict aerobes. Zirpolo (1927) found that *Vibrio pierantonii* would grow without luminescence in absence of oxygen. Doudoroff (1942) studied the anaerobic metabolism of *Achromobacter harveyi*, *Photobacterium fischeri*, *Ph. splendidum*, *Ph. seipiae*, and *Ph. phosphoreum* and detected the formation of formic, acetic, lactic, and succinic acids and alcohol, acetylmethylcarbinol and CO_2 . *Ph. phosphoreum* produced hydrogen and occasionally 2, 3-butylene glycol. Little if any ammonia was formed.

It was observed by Shoup (1928) that *Bacillus fischeri* could be preserved in glass tubes for years if inoculated on culture media and sealed in absence of oxygen (a hydrogen atmosphere). They would not grow under those conditions but when air was admitted, rapid growth and luminescence occurred.

Luminous Bacteria as Tests for Oxygen. Beijerinck (1902) was the first worker to utilize the luminescence of luminous bacteria as a test for oxygen. In his classic paper, "Photobacteria as a reactive in the investigation of the chlorophyll-function," he described the detection of oxygen formed by photosynthesis in an extract of crushed clover leaves to which 3% salt and luminous bacteria had been added. After the mixture (in a stoppered bottle) had become dark, due to the respiratory activity of the bacteria, an exposure to sunlight or even the lighting of a match was sufficient to cause luminosity of the bacteria. Beijerinck noted that if the mixture was allowed to stand for some hours the power of decomposing CO_2 was lost. He also tested crushed algae, which showed rather feeble photosynthesis, and a strip of the green alga, *Ulva*, exposed to the spectrum of a carbon arc. Photosynthesis occurred only in the red region. With a red alga, *Porphyra vulgaris*, the greatest photosynthesis was in the orange. He also demonstrated by lumi-

nous bacteria that oxygen passes from leaves of land plants through the stomata, rather than diffusing from both surfaces of the leaf.

A little later, Molisch (1904, 25) reported that the sap of green leaves, if chloroplasts were present, and also leaves carefully dried and powdered and suspended in emulsions of luminous bacteria could photosynthesize when illuminated. The author (1928) has used the method in classroom demonstrations and to establish the fact that 14 species of marine algae can start photosynthesis when illuminated in complete absence of oxygen after exposure to the anoxic conditions for 45 minutes. The eel grass (*Zostera marina*) was apparently injured by the lack of oxygen, as little photosynthesis occurred after 15 minutes anaerobiosis. Kostra (1928) has discussed bacteria in general as oxygen indicators.

Other instances of oxygen detection by luminous bacteria are numerous. Hill (1928) used luminous bacteria to demonstrate the diffusion of oxygen through rubber, wet collodion, paraffin, and oils (paraffin oil, vaseline, olive oil) often used to keep oxygen from liquids below them. Dry collodion, a thin film of de Khotinsky cement and medium motor oil were impervious to oxygen, but most oils allow it to pass readily. Sommenschein (1931) has recommended these bacteria as both indicators and oxygen consumers in growth of such strict anaerobes as tetanus and botulinus bacilli. The experiment of Johnson and van Schouwenburg (1939) with catalase decomposition of H_2O_2 in absence of oxygen has already been described.

The question arises as to the minimum concentration of oxygen visually detectable by use of luminous bacteria. The value cannot be too exact as the dark adaptation of the human eye varies and the minimum detectable light is dependent on the area emitting, the thickness of the bacterial suspension and other factors. Harvey and Morrison (1923) used flow meters to mix hydrogen containing a known small amount of oxygen with completely oxygen-free hydrogen, which then passed through the bacterial suspension. The apparatus was constructed with special precautions to prevent access of even traces of oxygen. The value found for just visible luminescence was less than 1 part oxygen in 100,000 parts hydrogen, about 0.0007 mm Hg.

Meyer (1942) has published a value 100,000 times smaller, one part in 10^{10} . His method involved the electrolytic formation of small amounts of oxygen in a neutral gas like nitrogen which was then passed in a fine stream of bubbles through the bacteria.

Despite the above results, it has been claimed by Richter (1926) that bacteria isolated from herring of the Baltic sea could luminesce

"ohne Sauerstoff" in the bottom of agar tubes with NaNO_3 but not with NaCl . Richter thought the bacteria might obtain oxygen from NaNO_3 but some exceptions were noted and his final conclusion was that they grew in media "praktisch O-frei."

Mudrak (1933) has also attributed the light in deep cultures to small amounts of dissolved oxygen. He could obtain no evidence that nitrates or chlorates supply oxygen for luminescence but thought they

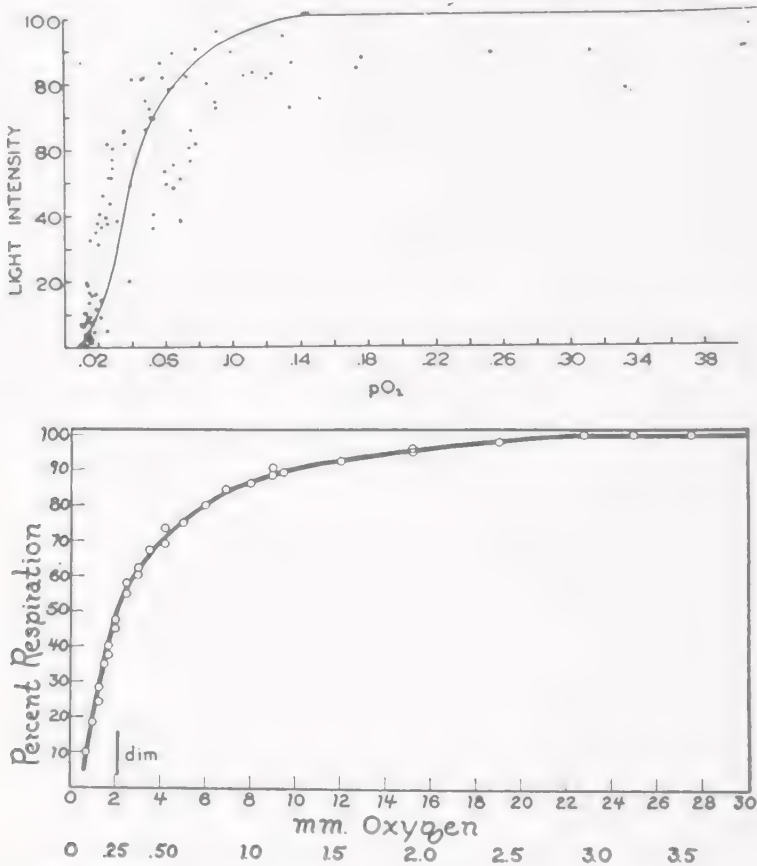


FIG. 6. The relation of per cent light intensity (above, after Shapiro) and per cent respiration (below, after Shoup) to oxygen pressure, expressed as per cent of an atmosphere. In lower figure, the millimeters of mercury oxygen pressure are also shown.

might be used in respiration, thus saving traces of dissolved oxygen in the medium that might support luminescence.

Luminescence and Oxygen Pressure. Since the development of photocells, a determination of the light intensity-oxygen pressure curve offers no special difficulties and Shapiro (1934) has investigated the relationship for the fresh water *Vibrio phosphorescens*. The curve is reproduced as Fig. 6. Light intensity is independent of oxygen pres-

sure until the latter becomes about 0.14% (1.06 mm Hg) when dimming begins and the light intensity falls off rapidly with decreasing oxygen pressure. The photocell-amplifier failed to respond at about 0.01% oxygen (0.076 mm Hg). The curve could be explained by adsorption of oxygen on the luciferase surface as a controlling factor in the activity at various oxygen pressures.

Respiration and Oxygen Pressure. The rapid utilization of oxygen by a suspension of luminous bacteria in a test tube has supplied a simple method of measuring oxygen consumption. By selecting a proper density of the bacteria and by making certain that the suspension is saturated with air at atmospheric pressure and is left undisturbed at constant temperature after saturation, the respiration may be measured by recording the time necessary for the suspension to become dim. This "dimming time" is proportional to the number of bacteria and the amount of dissolved oxygen and inversely proportional to the rate of respiration. It has been used by de Coulon (1916), the author (1925, 28), Taylor (1932, 34) and Root (1934) as a convenient method of testing the effect of various substances on the general respiration in relation to a control. KCN, for example, greatly prolongs the dimming time by decreasing the oxygen consumption. A check on this method (Harvey, 1928), by comparison with oxygen consumption measured by the Thunberg-Winterstein microrespirometer indicated good agreement. For a marine form (probably *Photobacterium phosphorescens*) the value was (at 21.5°C) 4.26×10^{-11} mg O₂ per bacterium per hour or 2.5×10^4 mg per kilo and 5.6 mg per square meter of bacterial surface per hour. The Q_{10} value (mm O₂ per milligram dry weight per hour) when oxygen and food are abundant is about 18 at 21°C. Johnson (1936) found the Q_{10} to be 4 for well-washed bacteria at 25°C and to increase four or five times when food material is present. Luminous bacteria are so small that diffusion of oxygen into the cell never becomes a limiting factor in respiration, as in the case of large tissues where internal regions may not obtain sufficient oxygen by diffusion.

The first study of oxygen consumption of luminous bacteria in relation to oxygen pressure was made by Shoup (1929), who used colorimetric and manometric (Thunberg-Winterstein microrespirometer) methods with a marine form and obtained the curve shown in Fig. 6. It will be noted that the oxygen consumption begins to fall off slowly when the oxygen pressure is reduced to 3% (22.8 mm Hg) and then more rapidly. The respiration is reduced one-half at 0.26% (2 mm Hg), at which point the light just begins to decrease. The curve approximates that of Langmuir for adsorption of a gas on surfaces and suggests that covering of the respiratory catalysts with oxygen de-

termines the rate of respiration. Above 3% oxygen the surface is saturated. In pure oxygen there is an irreversible reduction of the respiratory rate, apparently due to a poisonous action, which should be further investigated.

The decrease in respiration in pure oxygen has also been observed by Claren (1938) with *Micrococcus cyanophos*, but in this case the effect was reversible. It also depends on the type of nutrient present. With glucose, succinate and glycerine, which have little effect on respiration in different concentrations, the pure oxygen inhibition is small while with lactate, whose concentration does affect the respiration, the effect is large. The oxygen appears to be adsorbed in excess and the substrates are displaced from the catalytic surfaces, depending on their affinity in relation to that of oxygen. The studies of van Schouwenburg (1938) on the effect of pure oxygen and other oxygen pressures on cyanide inhibition of luminescence and respiration will be considered in the next section.

BACTERIAL LUMINESCENCE AND CELL RESPIRATION

The relation between cell respiration and luminescence has been much discussed. Respiration is a rather general expression used for all those chemical reactions which require the absorption of oxygen, and oxygen consumption is used as a measure of cell respiration. In this sense luminescence is dependent on cell respiration.

However, there are many conditions (high temperature, low salt content, drugs) under which luminous bacteria grow and respire without emitting light, and such changing environmental conditions as temperature and oxygen pressure affect luminescence and respiration in the same direction but without a proportional effect. The temperature coefficients of luminescence intensity and respiration are quite different and at lowered oxygen pressure respiration may be reduced 50% before light intensity even begins to decrease.

Moreover, the addition of certain substances (urethane) in the proper concentration to a bacterial suspension will practically abolish the luminescence and may actually increase the oxygen consumption while other substances (KCN) will reduce the oxygen consumption to a very low value, without correspondingly affecting light intensity. It is in this sense that the author (1920) has spoken of luminescence as being independent of cell respiration. The formation of a photogen (luciferin) must be dependent on respiratory processes.

Probably the best conditions for studying luminescence independently of cell respiration is after a period of anoxia when luciferin has accumulated in the cell and the excess is oxidized with the well-

known flash of light. At other times there is a steady state in luciferin production and oxidation, which is affected by various conditions that influence the general respiration. One of the most striking effects is that of glucose, which immediately increases both light intensity and respiration when added to bacteria washed free of nutrient material. A great deal of information has come from a study of respiratory poisons, particularly cyanide. The author (1932) has pointed out that cyanide experiments give conclusive proof of a relation between cell respiration and luminescence and has taken the position that "in the simplest organisms, the bacteria and the fungi, light production evolved in connection with the respiratory mechanism by the development of one of the hydrogen acceptors whose oxidation gives sufficient energy to excite a compound similar to luciferase." The excited molecule may not be luciferase, but there is no doubt of the intimate relation between respiratory processes and luminescence.

It is most important to know how much of the oxygen absorbed by a luminous bacterium goes into the light-producing reaction. The problem was undertaken by Eymers and van Schouwenburg (1936, 37) who analyzed the effect of various concentrations of KCN in reducing the oxygen consumption and the luminescence intensity. By plotting per cent reduction in oxygen consumption at various KCN concentrations, it was found that above 0.0003^{15} KCN the light intensity is linearly related to oxygen consumption, whereas below this concentration the oxygen consumption is much larger than the linear relation would imply, due to the fact that certain respiratory processes are not completely inhibited by cyanide. By extrapolation of the straight line relation between light intensity and oxygen consumption at different KCN concentrations to the oxygen consumption axis a value of 20% at 9.1°, 18% at 16.1°, and 19.5% at 22° was obtained. Eymers and van Schouwenburg regarded this approximately 20% as the oxygen consumption involved in the light-emitting process. There was always a small uninhibited oxygen consumption, 5 to 8% at different temperatures, even in high cyanide concentrations—the cyanide-insensitive respiration, observed with other cells. This was called the "rest" respiration and was considered connected with the yellow respiratory enzymes. The remaining 72 to 75% of the oxygen consumption was attributed to hemin respiration, sensitive to cyanide. These results are shown in Fig 7.

The continuation of these studies by van Schouwenburg (1938) has led to some interesting observations on the per cent of total oxygen consumption used in the "rest," the light, and the hemin respiration under

¹⁵ Light was reduced 50% and respiration to 12%.

various conditions. It was found that in peptone-free media a somewhat larger proportion (24%) of the oxygen consumption was used in the light respiration, with 7% "rest" and 69% hemin respiration.

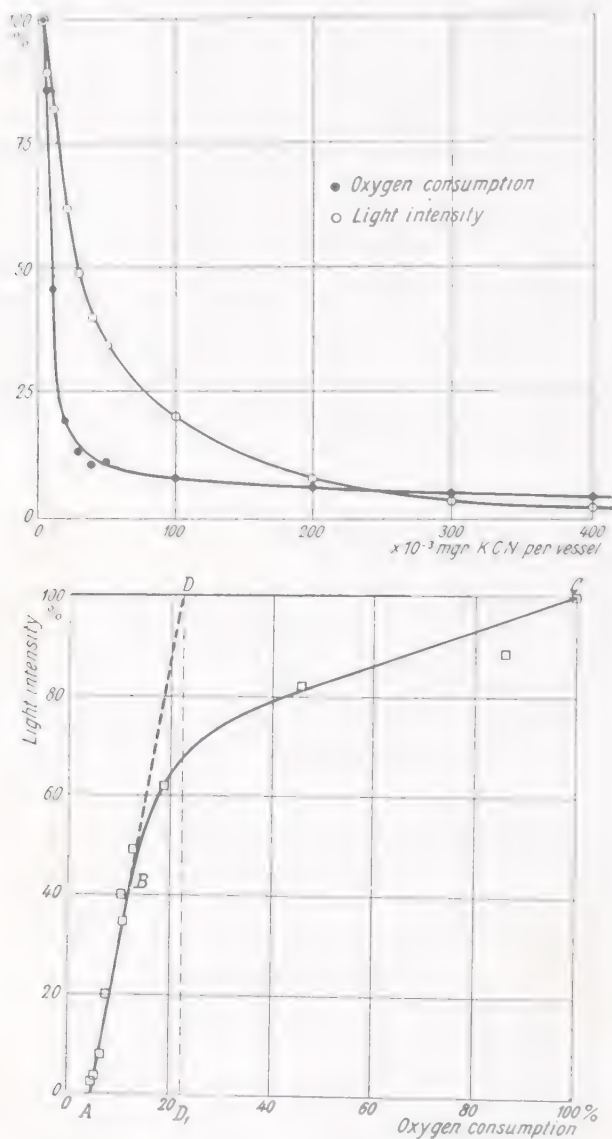


FIG. 7. (Above) Inhibition of oxygen consumption and light intensity of *Photobacterium phosphoreum* by KCN at 16.1°C. (Below) The above data plotted as light intensity vs. oxygen consumption. After Eymers and Van Schouwenburg.

Addition of substrates like glycerine to peptone-containing suspensions of bacteria increased both light intensity and oxygen consumption and when the added substrate was exhausted the oxygen consumption fell. If the medium was peptone-free, at the moment of exhaustion of

substrate the oxygen consumption fell but the light intensity increased momentarily and then decreased. The effect might be connected with autolytic processes, which start in absence of substrate.

The most important observation had to do with an increase of light intensity when cyanide was added to a suspension suffering from lack of oxygen. The explanation appeared to be connected with the cyanide inhibition of the hemin system, allowing the small amount of oxygen available to be used in the light-emitting process. That a competition for oxygen among the various oxygen-consuming systems exists is indicated by the effect of oxygen pressure on light production and total oxygen consumption in presence of cyanide. An exhaustive series of experiments led van Schouwenburg to the conclusion that, despite a constant oxygen consumption at various oxygen pressures there is a shift in the per cent used by various oxygen-consuming processes. The following tabulation shows the per cent of the total respiration used in three ways.

Per Cent of Oxygen in Gas Phase	Rest Respiration	Light Respiration	Hemin Respiration
5	10.6	10.8	78.6
10	10.8	13.2	76.0
21	11.5	21.3	67.2
50	8.0	70.5	21.5
100	11.0	89.0	0.0

It will be observed that the cyanide-insensitive rest respiration remains the same while in pure oxygen the hemin appears to be deactivated and hemin respiration does not occur.

These studies do not agree with the idea that all the oxygen used in the "light respiration" is actually involved in a reaction leading to light emission. Part of the oxygen may be involved in a reversible oxidation of luciferin without light production, and a rather insignificant part in the irreversible oxidation with light emission, a condition similar to that found by Anderson (1936) for *Cypridina luciferin*.

In favor of this postulate, van Schouwenburg found that after the hemin system had been completely inhibited by cyanide, addition of ethylurethan would inhibit the light emission without change in total oxygen consumption. This finding is in agreement with the idea that a negligible amount of oxygen is used in the light-emitting process itself. The action of the urethane appeared to be directly on the enzyme luciferase.

The masterly analysis of the complicated oxidative processes in the bacterial cells was summarized by van Schouwenburg as follows:



The hemin respiration is represented by I, the light respiration by II and III, of which only III is actually concerned in light emission. The rest respiration is not shown. Further information on the validity of the above scheme and the discovery of complicating factors has come from a study of the "flash" which occurs when air is readmitted to luminous bacteria after a period of anoxia.

FLASH KINETICS

The momentary increased brightness of luminous bacteria which occurs after they have been deprived of oxygen and air is readmitted

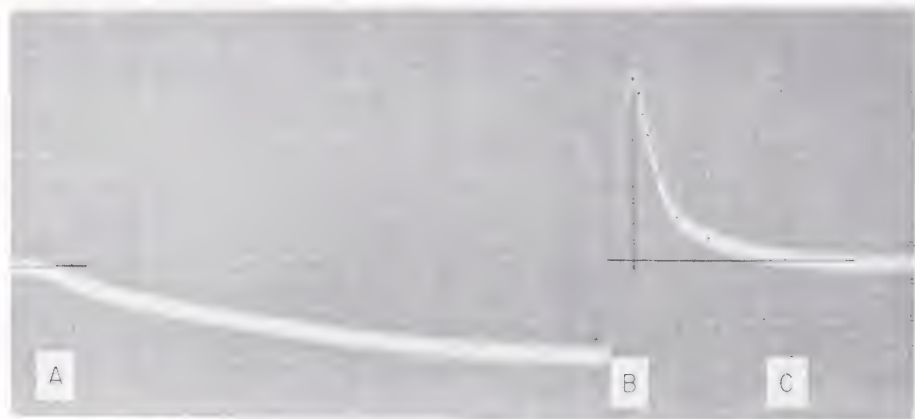


FIG. 8. A string galvanometer record of the light of luminous bacteria as they start to use up their own oxygen (A) and become completely dark (B). At B, more oxygen is admitted, and a flash or burst of luminescence ("excess luminescence") follows, with a return to the original steady light (C). Time (horizontal) is 12 seconds from A to B and 4 seconds from B to C. Light intensity vertical. Original record.

has been observed by a number of workers. It was first measured by the author (1932), who found that the flash might represent an increased brightness, nearly twice that of the normal value for the bacteria, and might last three to five seconds, as shown in Fig. 8. The total light emitted in the flash appeared to be independent of the duration of anoxia, provided these conditions were not too prolonged. This flash undoubtedly represents accumulation of luciferin which cannot be oxidized in absence of oxygen and offers an opportunity to study bacterial luciferin oxidation kinetics uncomplicated by accessory reac-

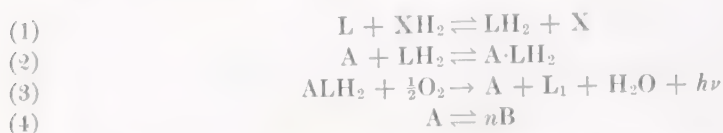
tions which maintain the steady-state luminescence observed under aerobic conditions. It is like having a mixture of bacterial luciferin and luciferase to which air is suddenly admitted and the development and decay of light can be studied.

It will be observed from Fig. 8 that the development of light is rather rapid, but the decay is slower and falls off more or less logarithmically as is the case after *Cypridina* luciferin and luciferase have been mixed. In this record the admission of oxygen to the bacterial suspension may have been a limiting factor in the form of the light development curve. In later records of Chance, Harvey, Johnson, and Millikan (1940) where special precautions were taken to introduce oxygen in approximately 0.001 second, the half time for light development is 0.08 second, a rather slow process compared with *Cypridina* luminescence where the half time is about 0.002 under the same conditions.

The flash has been extensively studied by Johnson, van Schouwenburg, and van der Burg, using *Photobacterium fischeri*. This form is unaffected by rather long periods of anoxia and returns to its original brightness when air is readmitted. On the other hand, *Ph. phosphoreum* is very sensitive to lack of oxygen which quickly reduces the normal light intensity, with rather slow recovery in air. This reduction in light intensity after short periods of anoxia in *Ph. phosphoreum* and long periods in *P. fischeri* was shown to be due to autolytic processes, in all probability to proteolytic decomposition of luciferase under anoxia. Not only the flash but also the steady state after anoxia are reduced, due to lowered luciferase concentration which is only slowly reconstituted. The effect is slowly reversible in presence of oxygen, provided the anoxic conditions have not been too prolonged.

The most important use of the flash technique is to demonstrate the formation of luciferin in presence of added materials. If well-washed and aerated bacteria, containing no food material, are deprived of oxygen, they give only a slight flash when it is readmitted; but if glucose is first supplied *anaerobically* and then oxygen is admitted, the total light in the flash is vastly greater. This experiment shows that anaerobic metabolism of glucose leads to the accumulation of luciferin. It was demonstrated that the increase in flash occurred with other compounds, like peptone and pyruvate, which can be metabolized in absence of oxygen but not with compounds like glycerol and succinate which require oxygen for metabolism. With glycerol and succinate, added *anaerobically*, the flash is small, but subsequently the steady state luminescence rises as these compounds are oxidized. Other experiments indicate that the glucose is not directly transformed into

luciferin but acts as a hydrogen donator to reduce the oxidized luciferin already stored up in the bacteria. The scheme of reactions which explain what is happening is as follows:



L , reversibly oxidized luciferin; LH_2 , luciferin, acting as an H-carrier; XH_2 , a hydrogen donator; A , luciferase; L_1 , irreversibly oxidized luciferin; nB , proteolytic decomposition products of A ; $h\nu$, a quantum of light.

This same flash technique has also been used to determine whether the effect of oxidative inhibitors in suppressing luminescence is directly on the luciferase or on some other oxidative process which in turn affects a steady state of light production in the delicate balance of reactions within a bacterium. For example it is well known that both cyanide and urethane decrease the light intensity of luminous bacteria in proper concentration. Do these substances act on the luciferin-luciferase system or on other oxidative enzymes in the cell? Johnson, van Schouwenburg, and van der Burg found that if luminous bacteria are kept in absence of oxygen and mixed with the proper amount of KCN anaerobically, when oxygen was readmitted, the flash represented the same amount of light as if cyanide had not been added. After the flash the subsequent level of light intensity decreased, as is regularly observed when this amount of cyanide is added. Hence luciferase, involved in oxidizing the excess of luciferin which has accumulated in absence of oxygen, is not affected by cyanide, which does attack other oxidations proceeding during steady state conditions.

On the other hand, urethane added under anaerobic conditions and then oxygen admitted results in a great decrease in flash intensity as well as a subsequent lower level of steady-state luminescence. Urethane certainly acts directly on the luciferase and may also act on other oxidative systems.

Schoepfle (1940, 41) has analyzed the kinetics of the flash of *Acromobacter fischeri* in detail at different temperatures and in the presence of veronal, dinitrophenol, and changes in osmotic pressure of the medium. Some records are reproduced in Fig. 9. The total light of the flash depends on the temperature at which the flash occurs, not on the temperature during anoxic conditions. The total light decreases with rising temperature, at least between 12 and 27°, a variation similar to that observed in the study of *Cypridina* luminescence kinetics.

The curves were interpreted in terms of the scheme of luminescence reactions proposed by Chance, Harvey, Johnson, and Millikan (1940) from studies of Cypridina luminescence. Two reactions, with velocity constants k_3 and k_4 , were assumed to be involved. One was the decomposition of a luciferase-luciferin-oxygen ($A \cdot LH_2 \cdot O$) combination to form excited luciferase (A'), oxidized luciferin (L), and water, with velocity constant, k_3 . The other reaction involves emission of a quantum of light from change of excited luciferase to the normal state (A) with velocity constant k_4 .

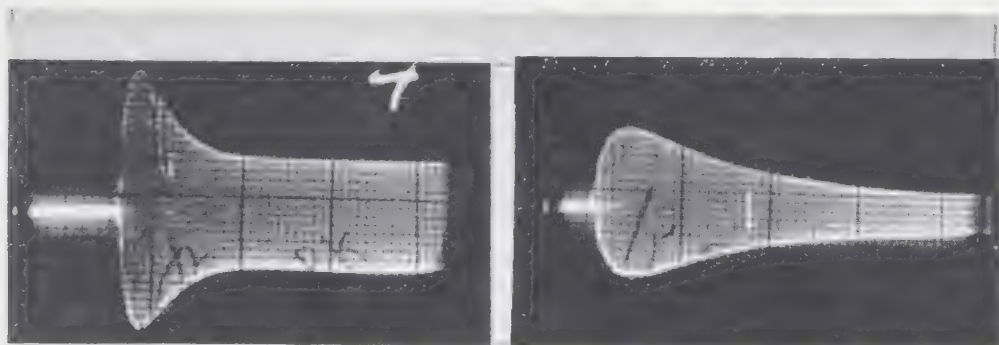


FIG. 9. The flash of luminous bacteria after anaerobic conditions, recorded by "A-C bridge unbalance" method. Photographs of the vertically oscillating electron beam as it traverses the screen of the oscilloscope from left to right. Curve at left was run at 23°C, at right at 12°C. After Schoepfle.

The first reaction results in an increase in concentration of A' and the second in a decrease. By expressing these reactions as two differential first order equations with rate of increase of A' proportional to $A \cdot LH_2 \cdot O$ and rate of disappearance of A' proportional to A' , taking their algebraic sum, and substituting light intensity I for rate of breakdown of A' , we have in integrated form:

$$I = \frac{k_3 k_4}{k_4 - k_3} \cdot (A \cdot LH_2 \cdot O)_0 [e^{-k_3 t} - e^{-k_4 t}]$$

where $(A \cdot LH_2 \cdot O)_0$ is the concentration at zero time.

Application of this equation to the actual curves at various temperatures gave the velocity constants, k_3 , and k_4 , and calculated points shown in circles of Fig. 10. The agreement is good. An Arrhenius equation plot of $\log k_3$ and $\log k_4$ versus the reciprocal of the absolute temperature is linear and indicates an activation energy of 8,000 calories for the $A \cdot LH_2 \cdot O$ breakdown (k_3) and no effect of temperature on the $A' \rightarrow A + h\nu$ breakdown (k_4).

Veronal and 2,4 dinitrophenol (in rather high concentration) and hypotonicity reduce the total light in the flash without changing constants k_1 and k_4 . Hypertonicity reduces the total light and in addition decreases k_2 , but there was no effect on k_4 . The decrease in k_2 may be attributed to increased viscosity within the bacteria as a result of exosmosis in the hypertonic medium.

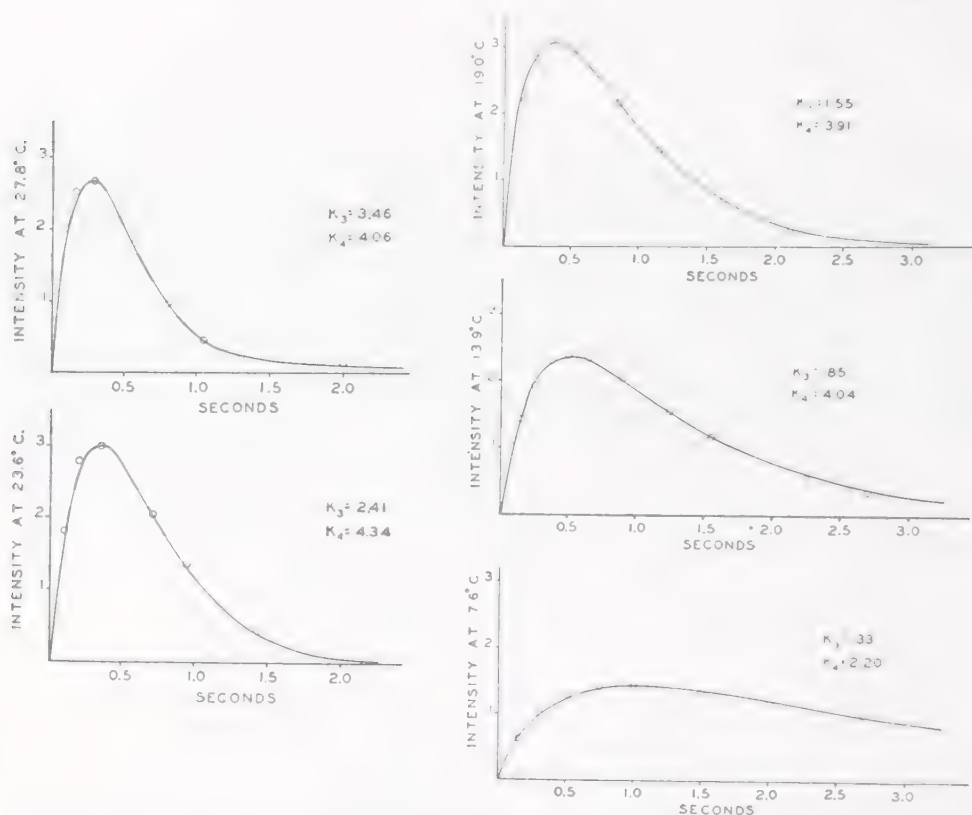


FIG. 10. Flash curves "corrected" for plateau intensity. The continuous curves represent actual experimental values. Open circles indicate points on the theoretical curve as calculated from the equation. After Schoepfle.

MECHANISM OF LIGHT EMISSION

In any chemiluminescent reaction, some molecule picks up part of the energy of the reaction, attaining an energy-rich or "excited" state, and later emits this energy as a quantum of light. In a direct chemiluminescence, one of the reaction products becomes excited; in an indirect chemiluminescence this excess energy is transferred to another molecule which then emits the light, analogous to the transfer of absorbed energy by a sensitizer molecule in a sensitized photochemical reaction (Taylor, 1927). Since the color of the light of fire flies ap-

peared to depend on the species supplying the luciferase, the author (1927) suggested that water molecules were primarily excited and transferred their energy to luciferase which then emitted the light. The idea of luciferase as the emitting molecule has been followed in a number of schemes (Harvey, 1935; Chance, Harvey, Johnson and Millikan, 1940; Schoepfle, 1940) for the mechanism of bacterial light emission. Since the fire-fly experiment can be explained in another way, and since, according to van der Burg (1943), it is very improbable that indirect chemiluminescences occur in solution, the question of the excited molecule is an open one. In the equations of page 44, no molecule has been designated as the emitter but in the papers of Johnson *et al.* (1945), Johnson (1947), and Johnson and Eyring (1948), the oxidized luciferin L^* (called excited luciferin) has been designated as excited and the following reactions have been postulated:

	With Luciferase	Additional Reactions that Occur with and Without Luciferase
(1)	$AL + XH_2 \rightleftharpoons ALH_2 + X$	(1') $L + XH_2 \rightleftharpoons LH_2 + X$
(2)	$A + LH_2 \rightleftharpoons ALH_2$	
(3)	$ALH_2 + O_2 \rightleftharpoons ALH + HO_2$	(3') $LH_2 + O_2 \rightleftharpoons LH + HO_2$
(4)	$ALH \rightleftharpoons AL^-H^+$	(4') $LH \rightleftharpoons L^- + H^+$
(5) alpha	$AL^- + LH \rightarrow AL^* + LH^- \rightarrow$ $AL + LH^- + h\nu$	(5') alpha $L^- + LH \rightarrow L^* + LH^- \rightarrow$ $L + LH^- + h\nu$
(5) beta	$AL^- + LH \rightarrow AL + LH^-$	(5') beta $L^- + LH \rightarrow L + LH^-$
(5) gamma	$AL^- + LH \rightarrow AL_1 + LH^-$	(5') gamma $L^- + LH \rightarrow L_1$
(6)	$ALH + O_2 \rightarrow AL + HO_2$	(6') $LH + O_2 \rightarrow L + HO_2$
(7)	$AL + O_2 \rightarrow AL_1$	(7') $L + O_2 \rightarrow L_1$

Symbols of components are the same as in the scheme of page 44. The existence of semiquinone formation (LH) and ionization are also shown.

Van der Kerk (1942) and Kluyver, van der Kerk, and van der Burg (1942) considered that the "complex of luciferase and irreversibly oxidized luciferin primarily appears in the excited state . . . and is responsible for the light emission," while van der Burg (1943) later suggested that possibly the light emission of bacteria is a "chemofluorescence," from transfer of energy to a pigment adsorbed on the luciferase, which is also sensitive to photochemical inactivation. Thus it will be observed that almost every possible molecule has been assigned the role of light emission by one or another student of the subject.

REDUCING ACTION

The ability of many bacteria to reduce a great variety of substances is well known. Beijerinck (1889) included this among three activities of luminous bacteria: (1) physiological combustion to which was due

the luminescence, (2) the reducing function, and (3) the fermenting function. *Photobacterium phosphorescens* as a facultative anaerobe was the only one of three species studied which could ferment sugar and produce hydrogen, while all three, *Ph. phosphorescens*, *Ph. luminosum*, and *Ph. indicum*, would reduce indigo although the reduction was slow when fermentation was proceeding. In 1904 he reviewed the ability of various forms to effect reduction and discussed the existence of enzymes which might be called hydrogenase and reductase but did not consider luminous bacteria specifically. Since Beijerinck's time many dyes have been used to study reduction and the evolution of hydrogen has been observed by other workers.

Methylene blue, made famous by the Thunberg technique, has been a favorite dye for study of reduction. It has been used by de Coulon (1916), Harvey (1929), Johnson (1937), Johnson and Chambers (1939), Nakamura and Fukumura (1949), and others to study various aspects of bacterial metabolism in relation to luminescence. Since the fundamental researches of Clark in the early 1920's and the recognition of a redox-potential scale, other dyes can be used more logically to express the reducing activity of cells.

The author (1929) made a preliminary survey of the ability of *Bacillus fischeri* to reduce a number of dyes, with especial reference to light emission in relation to dye reduction. Substances were studied whose redox potential at pH 7 ranged from +0.430 to -0.233 volt. The non toxic dyes could be divided into groups as follows: (1) dyes with potentials in the high oxidizing range (+0.233 to +0.217 volt), like orthochlor indophenol and 2,6-dichlor indophenol, which reduce to colorless compounds before the light disappears and reduce even while air is bubbled through the suspension; (2) dyes like orthocresol indophenol (+0.194 volt) in which the color partially disappears simultaneously with the luminescence and reduction is partial in air; (3) dyes like 1 naphthol 2SO₃ indophenol (+0.123 volt), where the color disappears simultaneously with the luminescence but if air is present no reduction occurs; (4) dyes like 1 naphthol 2SO₃ dichlor indophenol, methylene blue, and indigo tetra-, tri-, and disulfonate (+0.119 to -0.125 volt) which reduce only after luminescence disappears and remain reduced only in absence of oxygen; (5) finally, indigomonosulfonate (-0.182 volt), which was not reduced under the anaerobic conditions produced by the luminous bacterial suspension. In general the behavior is what might be expected from the position of the dye in the redox series. Some of the indophenols in groups 1 and 2 dim the luminescence when first added, but the luminescence intensity later returns. This was believed not to be connected with light absorption by

the added dye, but was explained as an initial rapid oxidation of luciferin by the indophenol, with subsequent production of more luciferin after the indophenol had been reduced.

Two substances of high oxidizing potential, not to be classed as dyes, have especial interest. They are potassium ferricyanide (+0.430 volt) and quinone (+0.278 volt). It might be expected that the former would rapidly oxidize the luciferin of luminous bacteria with consequent decrease of light intensity. However, no such effect was observed. The light intensity remained normal in $K_3Fe(CN)_6$, a behavior which the author ascribed to non-penetration into the bacterial cell. On the other hand, quinone quickly and irreversibly quenched the luminescence. This effect might be due to rapid oxidation of luciferin but is more probably to be attributed to toxic action—combination with luciferase—in a manner similar to some of the naphthoquinones, whose redox potential is in a region where they would be incapable of oxidizing luciferin.

The study of reducing activity was continued by Korr (1935), who measured the progressive change of potential in a culture through which nitrogen was bubbled, using platinum and gold electrodes. It is necessary to use a suspension with greater than a certain minimal concentration of bacteria to obtain consistent results. The pH was maintained at 7.6 and the temperature at 24–28°. In a buffered beefbroth-peptone fluid medium the general behavior of cultures is illustrated in Fig. 11. It will be observed that the lowest potentials attained were –0.214 volt, thus agreeing well with the –0.20 volt deduced from the dye reduction studies.

Korr made an extensive study of the effect of various conditions and agents which affect oxidative processes on the redox potential. These included temperature and such inhibitors as cyanide, urethanes, pyrophosphate, fluoride, iodoacetate, bromacetate, chloracetate, and arsenite. Space does not permit a detailed treatment of results, but in general there was a close connection between metabolic processes and potential which appeared to be determined by reaction rates of relatively positive and of relatively negative (dehydrogenase-substrate) systems. The reducing intensity was shown not to be due to cytolytic products.

The Thunberg technique has been made more accurate by Johnson (1937), who noted that, whereas washed luminous bacteria do not reduce methylene blue after six hours, the time is only 8 minutes with *m*. 10 glucose and 29 minutes with alpha-methylglucoside, a substance which can give up hydrogen to methylene blue but which inhibits aerobic respiration of washed cells.

Inhibition of the reduction of methylene blue in presence of sodium

diethylbarbital (veronal) was found by Johnson and Chambers (1939) to parallel the reduction in respiration rate (oxygen consumption) but not that of luminescence. Veronal in $0.01m$ concentration reduces luminescence some 20% with no effect on respiration and methylene blue reduction while higher concentrations decrease luminescence greatly with only slight effect on respiration and reduction. The veronal sensitive dehydrogenases appear to control the oxygen consumption but luciferase is not included among them and is not affected by small concentrations of veronal.

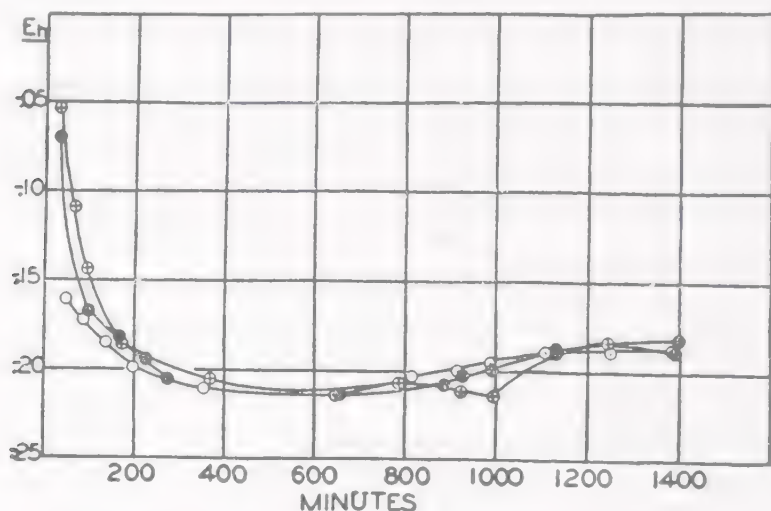


FIG. 11. Redox potential of suspensions of *Achromobacter fischeri* as a function of time. The various curves represent different concentrations of bacteria, all of them above the critical value necessary for reproducible results. After Korr.

A later study of dyes (in Japanese) has been made by Nakamura and Fukumura (1940) and Nakamura (1940), who have reported that *Micrococcus phosphoreus* and *Photobacterium phosphoreum* will luminesce under anaerobic conditions and in presence of dyes like indigo-carmin, capryl blue, cresyl blue, methylene blue, and thionine. Neutral red and Nile blue were without action.

RELATION TO pH

The need of a certain acidity or alkalinity for the growth of microorganisms was known to the earliest workers on pure cultures, but a rational expression of the facts and the development of an acidity scale had to await the work of Sørensen in 1909. It was noticed by Friedberger and Doepner (1907) that colonies of photobacteria growing near molds were much brighter than others away from the mold growth.

They attributed the effect to an alkaline medium and the excretion of substances favorable to luminescence.¹⁶

Hill (1928) studied the mold effect also and attributed it entirely to the alkalinity in the neighborhood of the mold, since otherwise acid production by bacterial cultures, insufficiently buffered, quickly results in a pH below 5.6, which kills the bacteria. He recommended addition of CaCO_3 to the culture medium to maintain alkalinity but found the CaCO_3 not as satisfactory as phosphate buffer itself. On a well-buffered medium, luminous bacterial cultures retained their light for a much longer time and can be transferred without a permanent loss of luminescence. Kostra (1928) discussed the behavior of luminous bacteria in relation to pH and Vouk, Skoric, and Klas (1931) give pH values 5.5 to 9.0 as the limits for a marine luminous bacterium.

The first attempt to measure the effect of pH on a luminous bacterial process was by Eymers and van Schouwenburg (1936), who found no difference in the emission spectra in phosphate buffers between pH 5.3 to 8 while van Schouwenburg (1938) observed the oxygen consumption of *Photobacterium phosphoreum* to steadily increase (from 164 units to 180 units) between a pH of 5.7 and 8.0 and that the optimum concentration of phosphate buffer was 0.07*m*. Clarens (1938) noted good respiration and luminescence of *Micrococcus cyanophos* between pH 5.9 and 8.3.

The effect of pH on light intensity in suspensions of *Photobacterium phosphoreum* has been studied by Johnson, Eyring, *et al.* (1945) in great detail. They found that different buffer systems and buffer concentration affected the light intensity at the same pH. Phosphate buffers allowed the greatest light emission while those containing phthalate were not quite so good and those with acetate reduced the light considerably. Giese (1946) has observed that borates reduce both luminescence and respiration of *Achromobacter fischeri* and should not be used in a buffer mixture.

The pH effect is dependent on temperature and pressure, and the curve at the optimum temperature of the bacterium and atmospheric pressure may be regarded as standard. In order to obtain a pH-luminescent intensity curve, it is necessary to take readings after a certain time interval, since there is a tendency for the light intensity to increase after it has been reduced to a minimal value by a change in pH. The

*Nadson (1968) noticed that when luminous *Photobacterium tuberosum* was grown together with *Micrococcus candicans* the cultures developed more slowly and the light appeared later. The effect might be the result of acid production but further studies are lacking. Haga (1944) has found a weak antagonistic action of *Microspira phosphoreum* against *Staphylococcus*, not connected with pH changes.



light is at a minimum one or two minutes after the change, and these readings have been plotted to give the curve for *Ph. phosphoreum* at the optimum temperature, 22° as shown in Fig. 12.

At the optimum temperature, the effects of changing pH are largely reversible over the range 3.6 and 8.8, but under more acid or more alkaline conditions destructive effects are evident. At higher than optimal temperatures, destructive effects are apparent at all pH values, but are slowest between pH 6.5 to 7. Below the optimum temperature

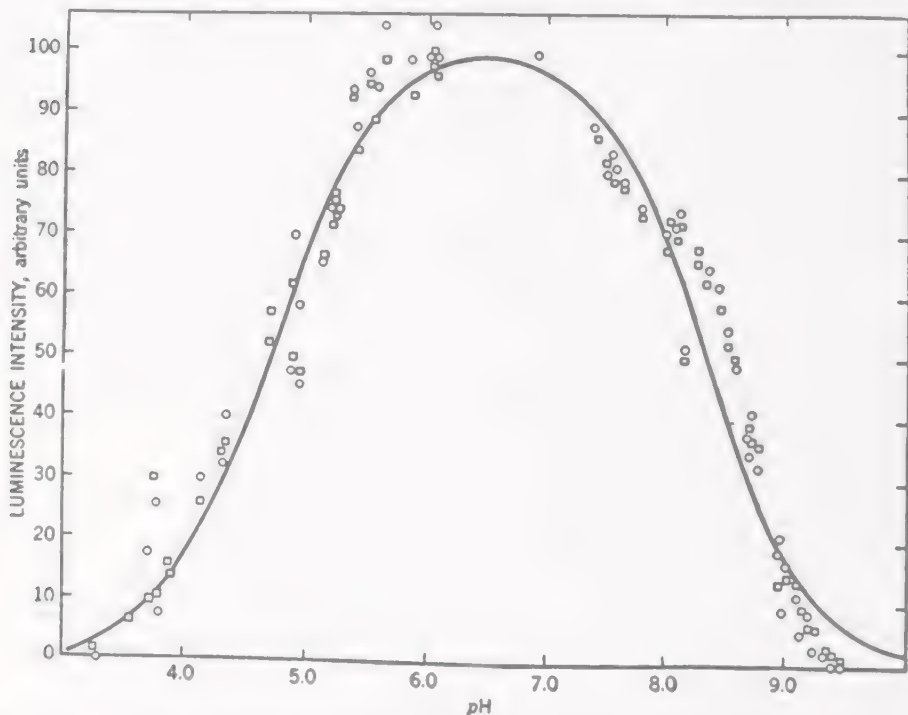


FIG. 12. Luminescence intensity of *Photobacterium phosphoreum* as a function of pH, determined 1 minute (squares) and 2 minutes (circles) after adding acid or alkali. After Johnson in *Advances in Enzymology*, Interscience Publishers, Inc.

the per cent decrease in light intensity at a given pH is much greater than at the optimum and greater the lower the temperature. There is also a slight shift of the optimum luminescence toward higher temperatures at low pH values.

The pH-luminescence intensity curve can be fitted by an equation similar to that for the relation between inhibitors and concentration of a type I inhibitor like sulfanilamide. The H ions in the acid range and the OH ions in alkaline range may be supposed to combine with the luciferase, eliminating its catalytic activity. A full mathematical treatment will be found in the Johnson, Eyring, *et al.* paper.

OSMOTIC PRESSURE

It was noticed by the early workers that marine fish often became luminous while fresh water fish did not unless they were sprinkled with salt solution. After culture methods had been developed for growth of luminous bacteria, two types were recognized, those requiring a fairly high salt content (marine forms) and those requiring little salt (originally isolated from fresh water material). The latter can withstand long contact with distilled water although they cannot grow in a salt-free medium. As early as 1889 Lehmann and later Nadson (1908) observed that cultures of *Photobacterium tuberosum* on salt-poor (0.5% NaCl) media allowed some growth but a very late appearance of luminescence. Issatschenko (1911) found that *Bacterium hippamis* would luminesce equally well on 0.5% and 3% NaCl.

The light of marine luminous bacteria disappears in fresh water and cannot be revived on adding salt. These marine forms undergo what may be termed "cytolysis" in distilled water and lose proteins and other substances from the cell. There is no doubt that the process is an osmotic one. It can be prevented by suspending the bacteria in pure sucrose of the same osmotic pressure as sea water but containing no salt whatever (Harvey, 1915). The effect of salt in maintaining osmotic pressure must be carefully distinguished from the effect in sustaining growth, respiration, luminescence, and other processes, which will be discussed in the next section.

Hill (1929) studied the "cytolysis" of marine luminous bacteria in connection with his investigation of the penetration of ammonium salts. He came to the conclusion that in hypotonic solutions and in freely penetrating solutions, the osmotic pressure of the bacterial contents caused the bacteria to swell slightly, but that they were surrounded by a rigid membrane which did not indefinitely stretch like that of an erythrocyte, but cracked as a result of pressure increase within, allowing the contents of the cell to escape. The same conclusions were drawn by Johnson and Harvey (1937) in an extended study of behavior in both hypotonic and hypertonic solutions, and the cracked cell membranes of cytolized bacteria can be observed in the electron microscope studies of Johnson, Zworykin, and Warren (1943) previously discussed. The effect on nuclear material and general cytological appearance of the bacterial cell has been described by Johnson and Gray (1949).

In hypertonic solutions a small amount of water is withdrawn from the cells, diminishing their volume at most some 27% when placed in 2.5 times concentrated sea water. That the cell membrane of bacteria

is very different from that of most animal cells is shown by their resistance to saponin (Lehmann, 1889) and also to Na glycocholate, which the author (1915) found to have no cytolyzing action and no effect on luminescence. No study has been made of the electrical charge of the bacterial surface but Sohngen (1913) found *Ph. phosphoreum* to be adsorbed on filter paper.

The influence of diluted and concentrated sea water on respiration and luminescence of *Achromobacter fischeri* has been studied by Johnson and Harvey (1938) and is reproduced as Fig. 13. It will be ob-

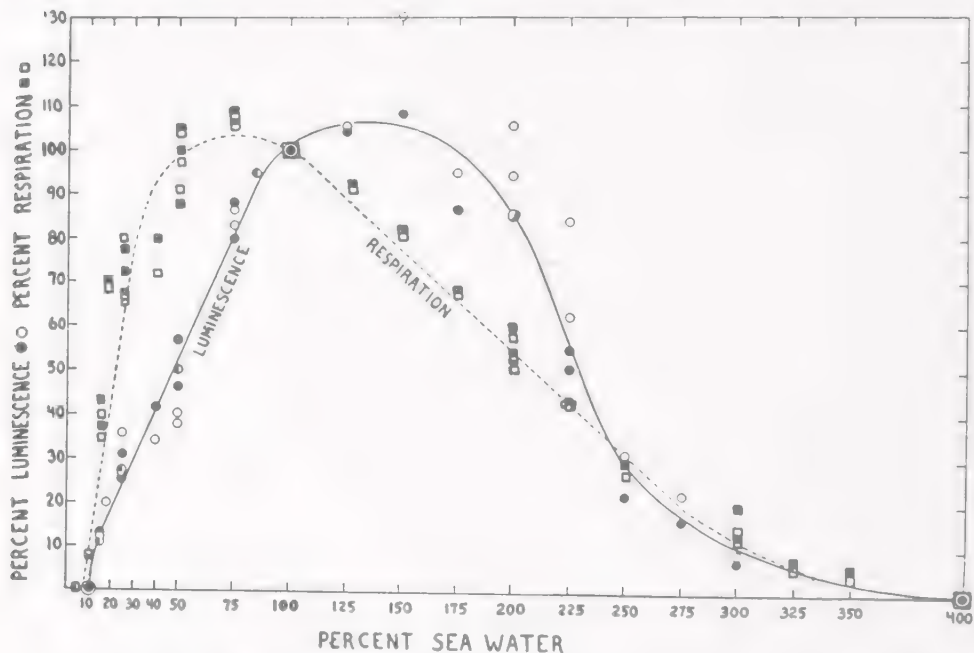


FIG. 13. The effect of diluted and of concentrated sea water on the luminescence (solid line) and oxygen consumption (dotted line) of the salt water luminous bacterium, *Achromobacter fischeri*. After Johnson and Harvey.

served that in moderately diluted sea water, luminescence falls off practically in proportion to dilution but respiration may continue at the normal high rate in 50% sea water. In concentrated sea water respiration falls off with increasing concentration but only in greater than twice concentrated sea water does the luminescence begin to be affected.

EFFECT OF SALTS

Most of the studies on salt effects have been made with marine forms, and the importance of NaCl for functional activity has been recognized by all workers, many of whom have studied the growth as well as luminescence. These bacteria are often remarkably independ-

ent of the kind of salt, provided the osmotic pressure is maintained, but the detailed results of the researches are so conflicting that it is not possible to draw very definite conclusions. The investigations have been mostly carried out by growing the organisms in culture media whose sodium chloride has been replaced by other salts, but in some cases the bacteria have been suspended in the salt solution, and any change in luminescence was noted.

Particular attention has been paid to salt effects by McKenney, 1902; Molisch, 1904, 12; Harvey, 1915; de Coulon, 1916; Gerretsen, 1920; Zirpolo, 1919-1923; Richter, 1926, 28; Lebenbaum, 1930; Fuhrmann, 1932; Mudrak, 1933; Bukatsch, 1936; Marcozzi, 1937; Claren, 1938; Johnson and Harvey, 1938; Takase, 1939; Yasaki and Kobayashi, 1946; and Yasaki and Kimura, 1946; and Farghaly, 1950. A brief summary of some of the results will illustrate the contradictory findings.

Molisch (1904, 12) found both growth and luminescence of *Bacterium phosphoreum* on a medium¹⁷ in which the chief salt was potassium chloride, iodide, nitrate or sulfate, sodium chloride, magnesium chloride, or calcium chloride, all in 3% concentration. With magnesium sulfate there was good growth but little light. K_2HPO_4 and $MnSO_4$ prevented growth and luminescence.

McKenney (1902) observed that *Bacillus phosphorescens* would grow and luminesce well in sodium chloride and magnesium chloride but not in potassium chloride, nitrate or sulfate, ammonium chloride, lithium nitrate, calcium chloride or nitrate, barium chloride, strontium nitrate, or rubidium sulfate while de Coulon (1916) found that *Pseudomonas luminescens* could grow and luminesce well on isotonic sodium chloride and nitrate, ammonium chloride, magnesium chloride and calcium chloride but not on potassium chloride or nitrate or sodium sulfate.

Zirpolo (1920, 21, 23) found that magnesium tartrate, sulfate, chloride and citrate increased growth and luminescence of squid bouillon cultures of *Bacillus Pierantonii* in concentrations up to 11%, the tartrate in concentrations up to 23%. KNO_3 increased the light intensity over a considerable range of concentration but cerium nitrate was toxic.

Gerretsen (1920) used *Photobacterium javanense*, replacing the sodium chloride of fish bouillon with sodium bromide, iodide, nitrate, sulfate, sulfite ($Na_2S_2O_3$), and acetate. Good growth occurred in all, and good light in all except iodide and acetate. When the sodium chloride is replaced by potassium, ammonium, lithium, calcium, mag-

The medium contained 10% gelatin, 2% sugar, 1% peptone, 0.25 K_2HPO_4 , and 0.25% $MgSO_4$.

nesium, and manganese chlorides, good light and growth occurred only in magnesium chloride, weak light in ammonium chloride. Potassium bromide, iodide, nitrate and sulfate, calcium nitrate and magnesium sulfate could not replace the sodium chloride.

Somewhat similar, but not exactly similar results were obtained by Richter (1926, 28), who found that Na could not be replaced by K or Mg, but that only small amounts were necessary. He considered Na so essential that he called it "Nahrsodium," and published a large paper on the subject. Lebenbaum (1930) also stressed the importance of Na, with K and Mg considerably less important, and Ca and SO_4 harmful. Studies on the importance of sodium were continued by Mudrak (1933), a pupil of Richter, using liquid media containing peptone or aspartic acid and glycerine as nutrients. Good luminescence occurred if sodium salts were present as chlorides, bromides, iodides, chlorates, nitrates, sulfites or phosphates but not with the corresponding potassium salts or in MgCl_2 or MgSO_4 .

On the other hand, Fuhrmann (1932) obtained good growth in bouillon cultures of glycerine or sugar with either sodium or potassium chloride and bromide, and Bukatsch (1936) made a careful study of the role of salts in a pure amino acid medium containing glycerine, finding that Na and K are essential but not Ca. A number of heavy metal salts were inhibitory but the action could be counteracted by Ca and Sr. Ba was less effective. Finally Marcozzi (1937) has claimed that Mg is necessary for the growth of luminous cultures and that unfavorable concentrations of Ca could be partially antagonized by Mg.

It is attractive to explain the divergent findings of different investigators as differences in the behavior of one or another species of luminous bacterium, but Mudrak studied ten halophile strains from salt water fish of the North Sea and the Baltic and one variety from veal and stated that they behaved alike. Possibly difference in concentrations and types of nutrient material are involved, but it is obvious that a great deal of painstaking research will be necessary to reconcile the various results.

The previous studies on salts have been carried out during the growth of the luminous bacteria in the salt-containing medium. Another method involves adding washed bacteria to the salt mixture and measuring the respiration and luminescence. Johnson and Harvey (1938) found the general respiration of well-washed suspensions of *Achromobacter fischeri* to be practically the same in isotonic sodium phosphate, sea water, and pure sodium chloride, but diminished in isotonic potassium chloride, sucrose, and magnesium chloride and prac

tically abolished in calcium chloride. Luminescence is more sensitive than respiration. It is almost quenched in calcium and magnesium chloride, greatly affected in potassium chloride, and somewhat affected in sucrose.

The results of Clarens (1938) are not entirely in agreement. He also used well-washed suspensions and studied respiration (but not luminescence) of *Micrococcus cyanophos* in different salt concentrations. Sodium chloride of 1.75% containing a little glucose proved to be the optimum concentration. In glucose solutions of the same osmotic pressure but without salt, respiration was about cut in half. The sodium could be replaced by lithium, potassium, or ammonium and the chloride ion by bromide or iodide without great changes in respiratory rate, but the ions did have an effect that ran parallel to ionic size. Replacing one-quarter of the sodium chloride with a phosphate buffer mixture ($\text{pH} = 6.98$) increased the oxygen consumption, and adding a little magnesium chloride to this had a still further beneficial effect which was not apparent when magnesium was added to sodium chloride alone. He found the relation of bacteria and salts to depend on the substrate and to be decidedly complicated.

Takase (1939) studied a symbiotic bacterium of high luminosity isolated from the luminous gland of the fish, *Coelorrhynchus kishinouyei*. A few bacteria were added to the salt solution, and the light was measured with a photocell and electrometer. NaCl, KCl, and KNO_3 were best for maintaining the light emission while Ca, Zn, and Pb were toxic, although NO_3 prevented toxic action of Ca.

Yasaki and Kimura (1946) and Yasaki and Kobayashi (1946) in Japanese papers have studied the effect on luminescence of various concentrations of NaCl and the relation of both growth and luminescence to concentration has recently been investigated for *Achromobacter fischeri* by Farghaly (1950), whose curve is illustrated in Fig. 14. Maximum growth appears at 1.5% NaCl while maximum luminescence occurs between 2.7–3.5% NaCl. Hence low concentrations of NaCl appear to prevent synthesis of the luminescent system. Preliminary experiments of Farghaly indicate that this inhibition is an osmotic effect, as restoration of osmotic conditions to those of normal (0.5 molar \approx 3% NaCl) by KCl, KNO_3 , K_2SO_4 , NaNO_3 , or Na_2SO_4 increases the luminescence 50% over the value in 2% NaCl.

It will be noted that both the growth and luminescence intensity studies of the various workers on salt effects disagree in certain particulars. The discrepancies may be due in part to different organisms used and in part to lack of care in removing traces of other salts and in

maintaining the proper reaction of the culture medium. Some of the salts are hydrolytically dissociated and may bring about marked changes in pH.

One of the interesting findings is that a single salt such as nitrate with various cations is as harmless as chloride. In fact the most striking result is the independence of luminous bacteria of salt antagonism, which is so important with most marine organisms. The author has

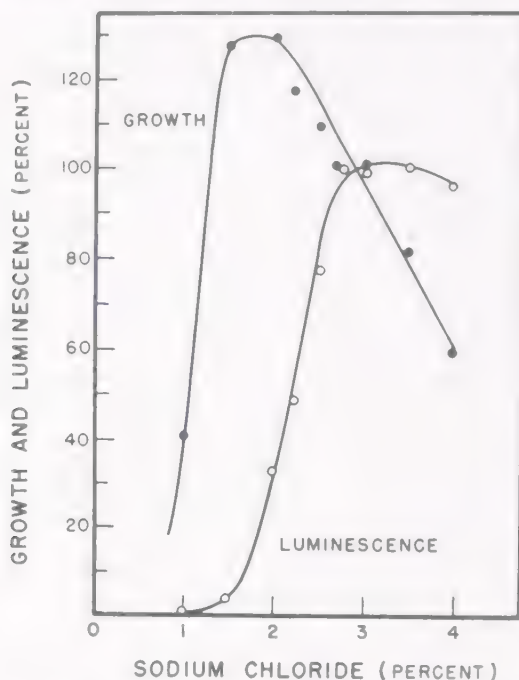


FIG. 14. The relation between growth and luminescence of *Achromobacter fischeri* and concentration of NaCl in the basal medium. The incubation period was 47 hours at 23°C. After Farghaly.

washed (1915) a suspension of luminous bacteria many times in pure sodium chloride containing no trace of calcium without ill effects or diminution of luminescence except that due to lack of nutrients.

PERMEABILITY

Relatively few studies on bacterial permeability have been carried out because of the small size of a bacterial cell. Since the marine forms undergo a change analogous to cytolysis, with loss of the ability to emit light, it is possible that their permeability to freely penetrating substances might be studied, using loss of luminescence as a criterion of penetration. This osmotic method of studying permeability has been placed on a firm quantitative foundation by Jacobs and his students.

The problem was undertaken by Hill (1929, 32), who concluded that luminous bacteria did in fact behave like erythrocytes and other cells. They cytolysed in ammonium salts of the fatty acids due to penetration of ammonia and fatty acid molecules which recombined within the bacteria, thereby increasing the osmotic pressure. Much slower cytolysis occurred in NH_4Cl and none in NaCl or sodium salts of the fatty acids. Hill found that in extreme pH ranges, injury effects mask the interpretation of penetration by loss of luminescence. Ammonia penetrates the bacterial cell with the greatest ease, while NaOH and HCl enter only after destruction of the cell surface.

EFFECTS OF RADIATION

Visible Light. Although a number of animals (certain dinoflagellates and ctenophores) lose their ability to luminesce in daylight or sunlight, luminous bacterial colonies are not as sensitive. Some observers have reported an inhibiting effect of light on luminous bacteria and others not. Dubois (1893) noticed that when *Photobacterium sarcoophilum* was grown in the dark the cultures remained clear and brightly luminescent, but in sunlight at 10° a yellow-orange color developed and the luminosity diminished, especially at the border of the colonies. Barnard (1899) also has stated that luminescence disappears slowly when luminous bacteria are kept in the light.

Suchsland (1898) found no effect of sunlight on *Bacterium phosphorescens*, kept under glass and water to prevent warming and McKenney (1902) also found no difference in the growth of *Bacillus phosphorescens* in darkness, alternate daylight and darkness, or continuously exposed to 16-candlepower electric lamp at 2 ft distance. Rizutti (1906) observed more rapid appearance of luminescence and a greater intensity in sunlight but this effect may have been due to increased temperature. On the other hand Lode (1908) reported that *Vibrio rumpel* was very sensitive to sunlight, Beijerinck (1915) found *Photobacter splendium* killed by direct sunlight, and de Coulon (1916) that green and violet light increased while rose and red light decreased the luminous intensity during growth. Morrison (1925) has reported that *Photobacterium phosphorescens* under polarized light grows faster and gives brighter colonies than under non-polarized light of the same intensity or in the dark, but the de Coulon and Morrison experiments should be repeated.

Since the above studies involved long-time exposures and growth of the bacteria, the author (1925) attempted to find some rapid inhibitory effects of light, analogous to inhibition of glowing mixtures of *Cypridina* luciferin and luciferase when exposed to the condensed beam

of a carbon arc which had passed through glass and water to remove radiant heat. Under these conditions the luminescence of the *Cypridina* mixture in a small test tube disappears in a few seconds in the region illuminated by the beam. However, no analogous decrease in light intensity of a dilute suspension of *Bacterium phosphorescens*¹ could be observed with the eye after six minutes, although the illumination was some 15,000 footcandles and the bacteria were examined a fraction of a second after the light was turned off. By using a phosphoscope no short time effects of illumination could be detected, at

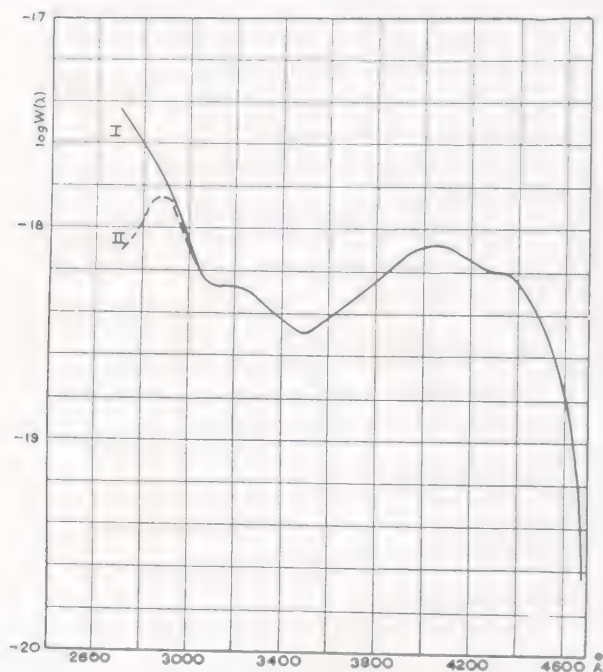


FIG. 15. The action spectrum for suppression of luminescence of *Photobacterium phosphoreum*. The log of specific photochemical effect ($W\lambda$) has been plotted vs. wave length. The dotted line is a correction for light scattering in the suspension. After Kluyver, van der Kerk, and van der Burg and Spruit.

least no decrease in luminescence was noted when examined 1/200 second after the illumination ceased.

It is possible that different strains of luminous bacteria behave differently, for van der Kerk (1942) and Kluyver, van der Kerk, and van der Burg (1942) observed inhibitory effects of light on *Photobacterium phosphoreum*. They found that over a wide range the product of light intensity by time of exposure to produce a given effect was constant and that scattering of light by the bacteria was not sufficient to interfere with measurement of an action spectrum for inhibition of luminescence, and "inactivation spectrum," as they called it. The curve is

¹ The author cannot vouch for this identification.

reproduced as Fig. 15, and it will be noted that some effect extends in the visible to a wave length of $480\text{ m}\mu$, with marked peaks at $408\text{ m}\mu$ and $290\text{ m}\mu$ and indications of secondary maxima at 320 and $430\text{ m}\mu$. Two other species of luminous bacteria, *P. splendidum* and *P. fischeri*, give a somewhat similar curve in the visible but extended only to $440\text{ m}\mu$.

An action spectrum is usually interpreted as the absorption spectrum of a compound involved in the process affected by the light. Van der Kerk and collaborators found that the photochemical effect was dependent on oxygen and most likely represented an action on what they call dehydroluciferin, i.e., reversibly oxidized luciferin. In presence of oxygen this compound would be produced in greatest concentration, thus reducing the concentration of luciferin. The action spectrum was quite different from the absorption spectra of flavins or carotenoid compounds, which might have acted as photosensitizers, and most closely resembled absorption spectra of the paraquinones and particularly the 1,4 naphthoquinone with a $\text{CO-CH}_2\text{OH}$ group directly substituted in the quinone ring, as in vitamin K derivatives. In a later study, however, Spruit (1946) concluded that luciferin, which he regarded as the corresponding naphthohydroquinone, was the molecule responsible for the chemical inactivation.

It was found by van der Kerk that in the visible region the light effect was reversible and the recovery had a monomolecular character. This was true in the ultraviolet also if the time of exposure was not too long, suggesting that the same substance was effected in both visible and ultraviolet. In the short ultraviolet (beyond $300\text{ m}\mu$) there were stimulatory and lethal effects which could be ruled out in the determination of the action spectrum.

Ultraviolet. The earliest work on ultraviolet light appears to be that of Dewar (1910), who found that luminous bacteria were killed even at liquid air temperatures. More extended work on ultraviolet from a quartz mercury arc was carried out by Beijerinck (1915) and Gerretsen (1915, 20), using *Photobacterium phosphorescens*. Gerretsen's curve for bacteria killed as a function of exposure is reproduced as Fig. 16. The effect of the ultraviolet is not immediate but the luminescence dims after a time and progressively fades until after some hours it may disappear.

Gerretsen occasionally observed a brighter luminescence after a short treatment with ultraviolet, a phenomenon also observed by van der Kerk and collaborators (1942). Gerretsen attributed this to liberation of oxygen by the culture medium and used the bacteria themselves to demonstrate the effect. A suspension of luminous bacteria in

a quartz tube is allowed to use all the contained oxygen and become dark. Then, after exposure of the tube to ultraviolet light, it can be observed that the regions exposed for a short time will luminesce. The author has confirmed this observation, but doubts if it can explain the stimulating effect of small doses of ultraviolet, since the light intensity of luminous bacteria is independent of oxygen pressure over a wide range.

Gerretsen attempted to identify the substance on which the ultraviolet acts. He found that the half of a plate of luminous bacteria exposed to ultraviolet still decomposed H_2O_2 and hence contained catalase, but the following day no catalase was present and the light had disappeared in the radiated half, although both light and catalase were

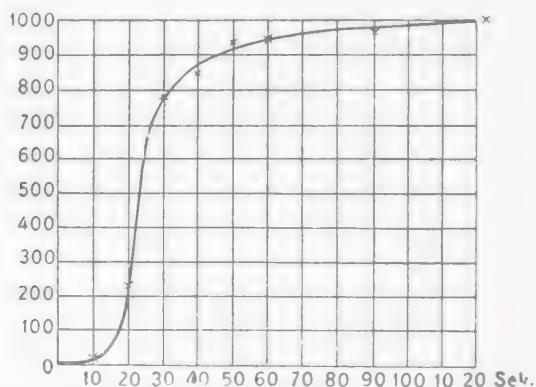


FIG. 16. Luminous bacteria killed (vertical) as a function of exposure (in seconds) to ultraviolet light. After Gerretsen.

present in the non-radiated half. The radiated half became brighter when glucose was added, just as did the non-radiated half. However, after 24 hours, there was abundant growth near the glucose on the non-radiated half, but no growth and darkness on the radiated half. He regarded the ultraviolet as affecting the growth process and not the luminescent process. Later work has indicated that luciferin is also photochemically changed, as indicated in the curve of van der Kerk and collaborators, whose conclusions regarding the action spectrum have already been presented.

Giese (1941) has also made a detailed study of ultraviolet effects on *Achromobacter fischeri*, measuring both luminescence and respiration. He found that small dosages which just prevent division of the bacteria do not affect respiration until some five hours have elapsed but larger doses immediately reduce the respiration and the effect is proportional to dosage. The ultraviolet acts on the bacteria, not on any

constituents of the culture medium and various experiments pointed to the dehydrogenases as the enzymes selectively affected. Survivors grown from a culture in which all but 1 in 10,000 bacteria had been killed, showed no greater resistance to radiation than controls. Luminescence decreases more rapidly after small doses of ultraviolet than does respiration, but the decrease of total light production was proportional to dosage and suggested a destruction of bacterial luciferin.

Spruit (1946) has corrected the action spectrum of van der Kerk for absorption by unspecific cell components in the far ultraviolet and also made an exhaustive study of the absorption spectra of various substituted naphthoquinones in oxidized and reduced condition.

X-Rays, Radium Rays, etc. There is probably no better material for radiation study than luminous bacteria, yet extended investigation of X-ray effects has not been made. Suchsland (1898) merely stated that X-rays do not affect *Bacterium phosphorescens*. A few observations on other types of radiation are to be found in the literature, and these should be repeated and extended, using the new sources now available. Koernicke (1904) observed that the light went out when near a weak radium source, but the bacteria were not killed. Omelianski (1911) exposed agar colonies of *Photobacterium italicum* to radium directly and through various types of screens. He found that weak doses stimulated growth and luminescence, while stronger doses inhibited. The effect was on the bacteria, not on the culture medium. The luminescence of a well-developed colony was not immediately affected, but only after some time, and an aluminum plate 0.5 mm thick prevented the lethal effect. Beijerinck (1916) also observed that radium and mesothorium radiation, like ultraviolet light, affected the reproductive function before that of luminescence, a condition which he described as necrobiotic.

The next paper was by Voormolen (1918), who used mesothorium and polonium, exposing the bacteria on agar covered with copper and a mica window. Growth occurred under the copper but not under the mica, until the mesothorium was removed, when growth started in that region. Voormolen believed the effect was due to the β -rays. Polonium emits mostly α -rays and also prevents growth, which does not begin when the polonium is removed.

Zirpolo (1920) studied the α , β , and γ rays from RaBr in a small glass tube hung in cultures of *Bacillus pierantonii*. He found that the luminescence was increased and lasted longer than in the controls. He also (1921) added uranium acetate and thorium nitrate to cultures of *Bacillus pierantonii* in various concentrations and found a toxic or inhibiting effect on growth and luminescence. Zirpolo was inclined to

attribute the effects to radiation but these salts could be toxic irrespective of radioactivity.

The work of Rerabek and Hykesova (1937) involved known quantities of radium emanation (4.5 millicuries), which saturated culture media inoculated with *Vibrio phosphorescens*. The effect was to reduce the light intensity throughout a period of days. Luminescence was affected more rapidly than growth, making these bacteria sensitive objects for detecting α - and β -rays.

Electrons, Ionized Air, etc. In a few unpublished experiments in 1947 with bursts of 3,500,000 volt electrons from the discharge of a capacitron,¹⁰ the author found that cultures of luminous bacteria in pyrex test tubes showed no immediate dimming, but that the light grew progressively weaker, and, depending on the number of doses, disappeared in a matter of hours. Subcultures could be grown from the irradiated bacteria, but the growth was slow and luminescence was delayed. An extensive study of electron effects and the action of other particles, for example, neutrons should be made.

Finally, the action of ionized air has been investigated by Hsu (1937), using *Coccobacillus coelorrhynchus*. He observed that there was no effect on growth of positively or negatively ionized air but that negative ions tend to increase the light while positive ions do not. This work should be repeated.

EFFECT OF HIGH-FREQUENCY SOUND WAVES

Like other bacteria, luminous species are broken up by high-frequency sound waves, if the conditions are such that cavitation occurs in the suspension. Harvey and Loomis (1929) found a *Bacillus fischeri* suspension in sea water to be gradually destroyed over a period of 90 minutes, with ultrasonic raying from a quartz crystal oscillator having a natural frequency of 400 kilocycles. The temperature was kept below 15, thereby ruling out heating effects. After 50 minutes' exposure the light was very faint and bacterial colonies did grow from inoculations on agar culture media but after 90 minutes the light was practically gone, and only one colony developed after inoculation. Korr (1935) found extinction of luminescence of bacteria in 15 to 20 minutes when using a powerful magnetostriction oscillator of 9,000 cycles.

EFFECT OF TEMPERATURE

In the preceding section dealing with the effect of light on luminous bacteria evidence was presented which suggested luciferin as the

¹⁰ The experiments were carried out in 1947 with the capacitron of the Electrolyzed Chemicals Corporation through the kindness of Dr. Wolfgang Huber.

particular substance undergoing photochemical decomposition. With far ultraviolet light, there were other more complex effects, which involved reactions important in the general respiration and growth of the bacteria and possibly also an alteration of luciferase. Since studies on the luciferin-luciferase reaction of the ostracod *Cypridina* have revealed that light intensity is a measure of the rate at which luciferin is undergoing a particular type of oxidation, it is obvious that this rate will be affected by change in several important factors. In a luminous bacterium, under steady-state conditions, where luciferin and luciferase are maintained constant and oxygen is present above a low minimal concentration, light intensity, I must be proportional to the active luciferase (An) and the luciferin (LH_2) concentrations and to the velocity constant k for this particular light-emitting reaction.

$$I \propto k(\text{LH}_2)(\text{An})$$

Various factors may change the concentration of LH_2 or An by preceding simultaneous or subsequent reactions. Rate of formation of luciferin from a precursor might be affected. Another particularly important effect is a change in active luciferase to the inactive or denatured enzyme (Ad), a change which may be reversible or irreversible. The magnitude of the above quantities may vary to a surprising extent among different species or strains of luminous bacteria.

One important application of the above equation has to do with the action of various substances such as drugs, which decrease light intensity by combining with luciferase, as in the case of pH effects already discussed. Another application involves the effect of temperature, which has been studied in great detail. It has been shown that rising temperatures increase k and that very high temperatures convert An to an inactive form.

The early workers on luminous fish and flesh noted that some light was emitted at the freezing point, that the intensity rose with increasing temperature to a maximum, and then declined and disappeared at slightly above body temperature. After the light of flesh was shown to be due to luminous bacteria, most of those who studied temperature merely recorded the temperature limits for their organisms. Values are given by Fischer (1888), Forster (1892), Lehmann (1889), Tollhausen (1889), Beijerinck (1889), McKenney (1902), Tarchanoff (1902), Molisch (1904), and Harvey (1913), ranging generally from -12 to 40° . Eijkmann (1892) placed the temperature limits at -20 to 45° . Lebenbaum (1930) saw a feeble light in a form similar to *Bacterium phosphoreum* at -25° , and Akabane (1938) in symbiotic bacteria from fish at -14° to 43° .

Very Low Temperatures. Although the light disappears at around -10° , Suchsland (1898) demonstrated that *Bacterium phosphorescens* would stand a temperature of -80°C and luminesce when again warmed. There appears to be no lower lethal limit. Macfayden (1900, 03), Dewar (1910), Harvey (1913) and Zirpolo (1929) have all found that the bacteria are not injured by a temperature of liquid air (-190°C) but on rewarming will luminesce and grow normally. The first two authors used *Bacillus phosphorescens* and *Photobacterium balticum*, exposed on strips of filter paper directly immersed in the liquid air (for 7 hours) or in small tubes (for 7 days). If the bacteria were ground at the liquid air temperature, no light appeared on warming. Macfayden and Rowland (1900) and Zirpolo (1932, 33) found a liquid hydrogen exposure to -252°C for 10 hours did not injure the bacteria. Zirpolo used *Bacillus pierantonii* in his liquid hydrogen experiments and also studied liquid helium effects when kept at -271°C for several days. The bacteria again luminesced when warmed. The disappearance of light at very low temperatures is undoubtedly connected with inability of the luminescent reaction to proceed in a non-liquid medium.

A general study of many photochemical effects at the temperature of liquid air was made by Dewar (1910), who was surprised to find that *Photobacterium phosphorescens* exposed for five minutes to ultraviolet light at liquid air temperatures did not luminesce on warming. Practically all the bacteria were killed and would not grow. Thinking that the action might be due to formation of ozone, which then killed the bacteria, Dewar repeated the experiment in a nitrogen or hydrogen atmosphere and found that the lethal action of ultraviolet was still marked not only on this particular luminous form, a facultative anaerobe, but also on other non-luminous bacteria. Dewar suggested that possibly electrically charged ions acting on their surface were the agents of destruction.

Very High Temperatures. The earliest observers found that the light of shining flesh, due to bacteria, disappeared when heated to well below the boiling point and would not return on cooling. The more careful experiments of Canton in 1769, quoted at the beginning of this chapter, and many later workers drew attention to the return of light, if cooled immediately after the light had disappeared. In fact the upper temperature limits for luminescence and the return of light on cooling are variable quantities, depending on rate of heating or time kept at the high temperature. There occur both reversible and irreversible changes which are characteristic of all bioluminescences and in fact of all living processes. Concerning high temperature effects on

light emission, the author (1920) wrote: "For a given mixture of luciferin and luciferase [of Cypridina] the light becomes more intense with increasing temperature up to a definite optimum and then decreases in intensity. The diminution in intensity above the optimum is due to a reversible change in the luciferase so that its active mass diminishes." A quantitative treatment of the effect at high temperature has been developed by Eyring, Johnson, and collaborators which can best be presented by a consideration of the whole luminescence-intensity-temperature curve.

The Luminescence Intensity-Temperature curve. The first quantitative measurement of luminescence as a function of temperature was made by Morrison (1925), followed by Root (1932), Akabane (1938), and Brown, Johnson, and Marsland (1942). Many later workers have published luminescence-temperature curves in connection with drug effects which are intimately related to temperature and have been extensively studied and fully reviewed by Johnson, Eyring, *et al.* (1945) and by Johnson (1947, 48).

Root (1934) also determined the effect of temperature on oxygen consumption and found that oxygen is still being absorbed at a temperature (35°) where the luminescence is greatly reduced, and that the temperature coefficients for luminescence and for respiration are quite different.

When plotted in the conventional Arrhenius form of log light intensity versus the reciprocal of the absolute temperature, a straight line is obtained only at temperatures well below the optimum. As the optimum is approached, the straight line deviates, showing "breaks." In Root's measurements with the fresh water *Vibrio phosphoreus*, the temperature coefficients (μ values) for luminescence were 20,000 to 27,000; for respiration 15,000 to 22,000 calories. Morrison's μ values for luminescence of *Bacterium phosphoreus* were 16,600 and 22,440, whereas Brown, Johnson, and Marsland obtained 17,000 calories for luminescence of *Photobacterium phosphoreum*.

As the temperature is increased above the optimum, the secondary effects of reversible and irreversible character appear, leading to decreased light production. Values above the optimum are not too exact due to the time factor. The longer the suspension is kept at the high temperature, the dimmer does its light become, and the less reversible on cooling. In Fig. 17 the dots indicate the intensity of a suspension kept at 35° for a few seconds and then cooled to 15° and to 7°.

The temperature-luminescence intensity curves of all luminous bacteria are similar to those of Fig. 17, but various species of bacteria

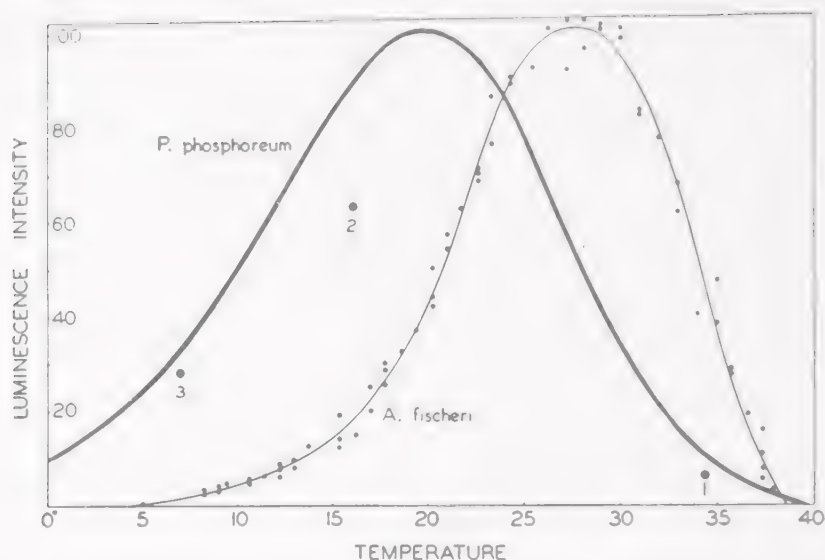


FIG. 17. Luminescence intensity as a function of temperature. The dots (1, 2, and 3) show intensity values after the suspension was kept at 35°C for a few seconds and then cooled. After Brown, Johnson, and Marsland.

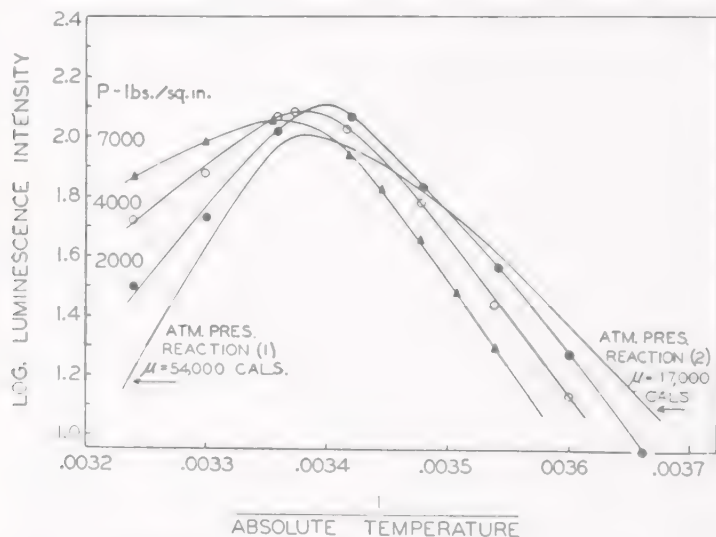
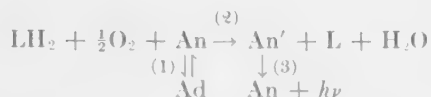


FIG. 18. The luminescence intensity-temperature relation for *Photobacterium phosphoreum* at the various pressures indicated. The log luminescence intensity is plotted against the reciprocal of the absolute temperature. After Brown, Johnson, and Marsland.

have their characteristic optima. The effects of temperature are intimately connected with those of pressure and the interrelationships have been fully worked out. The effects of pressure are illustrated in Fig. 18, taken from the paper of Brown, Johnson, and Marsland who explained the form of the curves as the resultant of temperature and pressure effects on the luminescent reaction and on the reversible equi-

librium between native and denatured luciferase. They proposed the scheme:



in which LH_2 = luciferin, An = active or native luciferase, An' = excited luciferase,²⁸ with sufficient energy to radiate. Ad = denatured or inactive luciferase. L = oxidized luciferin, and $h\nu$ = a quantum of light.

Increasing temperature increases the rate of reaction (2), has no effect on reaction (3), and changes the equilibrium between An and Ad , the reversible thermal reaction (1), in the direction of Ad . Below the optimum temperature, luminescence increases with temperature, primarily resulting from reaction (2) with a μ value of 17,000 calories, while above the optimum, intensity decreases in proportion to the reversible thermal inactivation of the enzyme by reaction (1) with a μ value of 54,000 calories. At intermediate temperatures both processes are important, their opposing effects exactly balancing at the optimum temperature. Curves similar to those for luminescence at different temperatures have been observed in a wide variety of physiological processes and the explanation is in all probability the same.

In connection with the effects of temperature on chemical reactions, an interesting question arises as to how quickly a new reaction rate will be assumed when the temperature is suddenly changed. Specifically, if the velocity constant for inversion of cane sugar has a certain value at 10° C and the temperature is instantaneously changed to 20° C, will the new constant be instantly assumed or will there be a delay? Luminous bacteria offer particularly advantageous material for study of this effect, since the light intensity is a direct measure of reaction velocity dx/dt and can be instantly recorded.

Kreezer and Kreezer (1947) have investigated this problem and shown that when luminous bacteria are quickly changed from one temperature to another below the optimum, the new light intensity is not immediately assumed but a lag does occur. The temperature was changed from 8.5° to 14.5° in one second while the light intensity took over three seconds to attain its new value. The curve was analyzed as a transient and was found to have a time factor of 0.72 second, and the delay could not be attributed to a lag in the transmission of heat through the bacterial membrane.

²⁸ It may be that L is the excited molecule, but this would make no difference in the end result, provided the change $\text{L}' \rightarrow \text{L} + h\nu$ was not affected by temperature.

EFFECT OF HYDROSTATIC PRESSURE

As early as 1898 Suchsland subjected *Bacterium phosphorescens* to 200 atmospheres for eight minutes and 230 atmospheres for one minute without finding an effect on luminescence, but the bacteria were not observed while under pressure. Actual observation of the effects of hydrostatic pressure on luminescence of bacteria has revealed some remarkable phenomena, first pointed out by Brown, Johnson, and Marsland (1942), that have led to a better understanding of many vital processes, particularly the action of drugs on cells. From previous studies of Regnard, Edwards, Cattell, Ebbecke, Brown, Marsland, Pease, and others it has been realized that increased pressures may result in a decrease or an increase in the physiological activity of a cell, depending on the activity studied. Increase of hydrostatic pressure may cause sol-gel changes in the cell or may change reaction velocities and equilibria.²¹

In an ameba, pressure effects on locomotion are due to sol-gel changes but in luminous bacteria, hydrostatic pressure affects luminescence intensity by affecting reaction rates and equilibria. It has been found that increased pressure decreases the light intensity if applied below the temperature optimum and increases it above the temperature optimum, as indicated in Fig. 18. It is a rather remarkable sight to observe a suspension of *Achromobacter fischeri* at 35°C, some 10 degrees above the optimum for this species, where the luminescence is greatly decreased, exhibit immediate return to optimal brightness when the pressure is increased to 6,400 lb per square inch. On releasing the pressure, the light intensity immediately decreases again. The actual experiment is illustrated in Fig. 19. A study of the same species of luminous bacteria reveals an immediate reversible decrease in luminescence intensity when a pressure of 6,400 lb per square inch is applied at 15°C, some 10 degrees below the optimum, and a return to the normal brightness at 15°C when the pressure is released.

These results can be explained by assuming that reactions (1) and (2), page 69, both proceed with an increase in volume and that pressure, by reducing the volume, slows both reactions. At low temperatures, pressure reduces the luminescence intensity by slowing the dominant reaction (2). At temperatures above the optimum, where reaction (1) is dominant, compression increases luminescence by reactivating the thermally denatured luciferase.

See the review on "Physiological effects of pressure" by McKeen Cattell in *Biol. Revs.*, 11, 441, 1936, and by D. A. Marsland in *The Structure of Protoplasm*, Ames, Iowa, pp. 127-61, 1942.

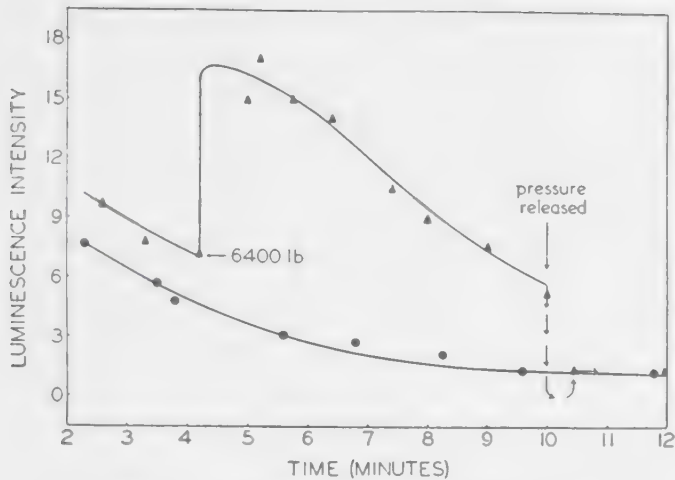


FIG. 19. The increase in luminescence under pressure, at 35°C. Lower curve shows irreversible decay in luminescence in control chamber, due to the high temperature. Upper curve shows increased intensity upon compression and the reversal by decompression. Irreversible decay of luminescence proceeds practically unchanged at the high pressure. After Brown, Johnson, and Marsland.

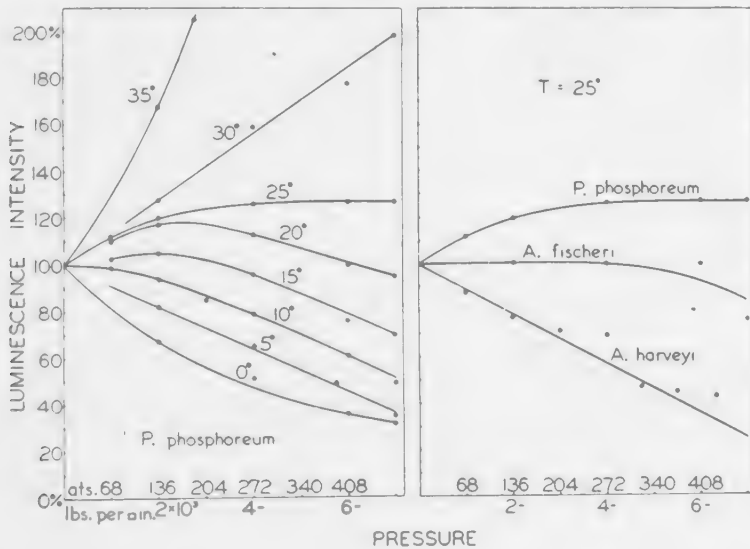


FIG. 20. Luminescence intensity and pressure. At left is plotted luminescence at different temperatures and at right luminescence at one temperature for three different kinds of bacteria.

The importance of the optimum temperature in the effect of pressure is illustrated in Fig. 20 which shows (left) the light intensity as a function of pressure in *Photobacterium phosphoreum* where optimum temperature is at 21° and (right) the effect of pressure at 25° on three bacteria whose optimum temperatures vary. At 25°, the optimum for *Achromobacter fischeri*, pressure has practically no effect, but pressure

increases the light for *P. phosphoreum* (optimum 21°) and decreases the light of *Achromobacter harveyi* (optimum at 32°).

The above explanation of temperature and pressure effects assumes that luciferase is the enzyme in the bacteria which is denatured by heat. A few preliminary unpublished experiments of Johnson and the author have indicated that luminescent mixtures of purified *Cypridina* luciferin and luciferase are affected by pressure at high temperatures as is the luminescence of bacteria. However, these studies indicate that pressure effects are complex and influenced by impurities in a manner which will take considerable research to elucidate. That luciferase is one enzyme in bacteria affected by temperature and pressure seems highly probable, but others may also be affected since the thermal denaturation of a number of pure enzymes and proteins have been shown by Johnson and collaborators to be reversed or retarded by pressure.

Application of the theory of absolute reaction rates (Eyring and Magee, 1942) to the data allows quantitative interpretation of pressure and temperature relations of bacterial luminescence, with prediction of results under varying conditions, and the calculation of such constants for the various reactions as the heat of activation, the energy of activation and the volume change of activation, as well as the constants of equilibria involved. Equations and details will be found in the papers of Johnson, Eyring, *et al.* (1945), Johnson (1947, 48), McElroy (1947), Johnson and Eyring (1948), and Eyring, Lumry, and Woodbury (1949).

ACTION OF DRUGS

Early studies on the effect of alkaloids and other drugs on bacterial luminescence were made by Lehmann (1889), who found that in many cases the light was not immediately affected. Beijerinck (1891) also studied many substances by his auxanographic method. McKenney (1902) and Ballner (1907) found reversible quenching of light with ether or chloroform vapor, Molisch (1912) with tobacco smoke and Harvey (1915) for a series of alcohols from methyl to capryl. The reversible inhibition of luminescence by alcohols was more marked the greater the number of carbon atoms. Ether and methyl and ethyl alcohol were also studied by de Coulon (1916) and hypnotics and alkaloids by Zirpolo (1920, 22). The general conclusion that ether, alcohols, etc., could reversibly decrease the light of the bacteria agrees with their effect on other physiological processes and indicates true narcosis. Too great concentrations always lead to death of the cells.

Modern research had added many new groups of narcotics, the urethanes and the barbiturates, and other types of compounds like sulfa drugs and antibiotics which make classification of the action of organic compounds rather difficult. The group of alkaloids and hormones which have specific effects in higher animals and certain compounds like the dinitrophenols, naphthoquinone, cyanides, arsenites, iodoacetates, and carbon monoxide usually thought of as respiratory inhibitors, also have very definite effects on luminescence.

Most of the above compounds have a special interest as they may frequently be used to analyze the process of light emission in relation to other reactions in the cell. Such an analysis has already been described for KCN in connection with the relation between luminescence and cell respiration. It is not possible to review in detail the effect of each specific substance but reference will be made to the literature describing the effect. Many compounds have been studied by Usami and Yokosuki (1945).

A number of generalizations have recently been drawn regarding drugs and luminescence of bacteria that are of great importance for the interpretation of the mechanism of drug action in general and the influence of temperature and pressure in particular. It has long been recognized that temperature modifies the action of drugs and, as early as 1901, Meyer²² divided narcotics into two groups, one acting more intensely at high temperatures, the other at low temperatures. The effect was so striking that, using a narcotic of the first group, tadpoles narcotized at a high temperature would come out of the narcotic and swim around if the temperature was lowered. In the case of a narcotic of the second group, tadpoles narcotized at a low temperature would recover when the temperature was raised. Meyer related narcosis to the lipid solubility of the narcotic, and the temperature influence was explained by the partition coefficients between oil and water at the different temperatures.

These temperature effects can also be observed for narcotics and drugs in their effect on luminous bacteria, and the action of the drug can be treated quantitatively and predicted from equations developed by Eyring, Johnson, and collaborators. The explanation does not involve lipid solubility and partition coefficients but combination of the drug with enzymes systems, thus designating them as inhibitors of certain processes within the bacterial cell. A review of the inhibition of cellular activity by narcotics has been published by McElroy (1947).

Pressure also affects the activity of the drug. From what has

²² H. H. Meyer, *Arch. exp. Path. Pharm.*, **46**, 338, 1901.

previously been said of temperature and pressure in relation to luminescence, it is of the greatest interest to find that hydrostatic pressure can completely reverse the inhibition of certain drugs at the optimum temperature where hydrostatic pressure (below 7,000 lb. in.) without the drug would have very little effect on luminescence. Johnson, Brown, and Marsland (1942) first observed this reversal by pressure

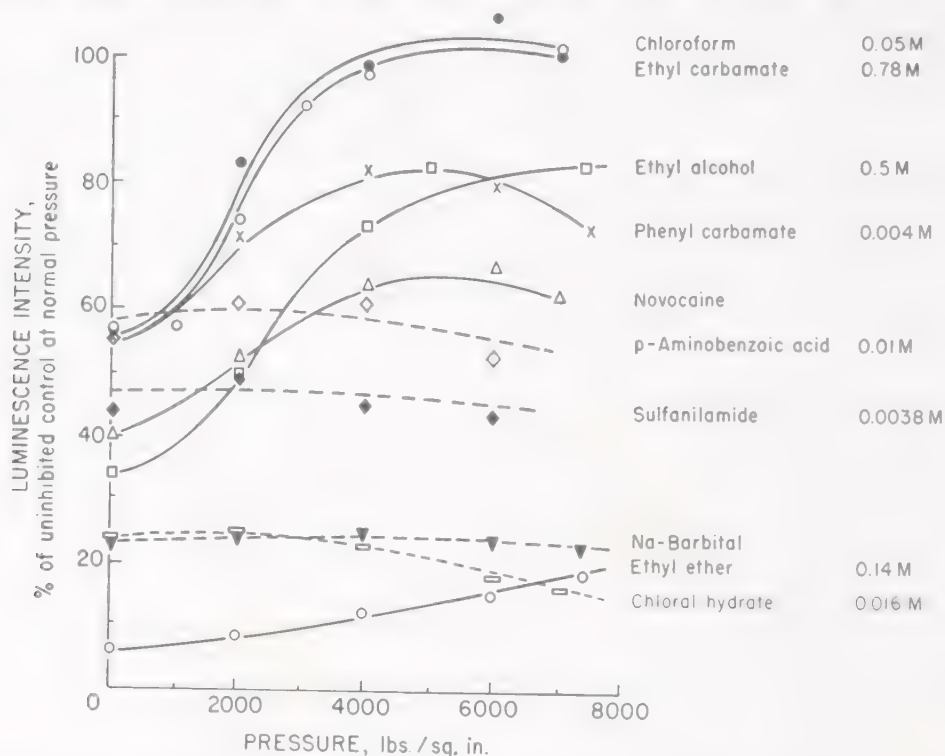


FIG. 21. The effect of hydrostatic pressure at 17° to 18°C on the luminescence intensity of *Photobacterium phosphoreum*, suspended in buffered salt solution plus various inhibitors indicated at the right of the curves. Intensity is expressed as per cent with respect to a control, without inhibitor, at atmospheric pressure. Hydrostatic pressure alone causes not more than a 10% difference in the control intensity of this species at this temperature. The curves for inhibitions not sensitive to pressure are indicated by dotted lines; the others by solid lines. After Johnson, Brown, and Marsland.

of the inhibitory effect of chloroform, alcohol, ethyl urethane, phenyl urethane, procaine and ethyl ether. They observed no reversing effect of pressure on inhibition by sulfanilamide, para-aminobenzoic acid, chloral hydrate, or barbital. Figure 21 shows the effects. Since then a number of other narcotics (McElroy, 1943, 44), quinine (Johnson and Schneyer, 1944), and a whole series of carbamates (Johnson, Simpson, and Flagler, 1950) have been studied.

Explanation of the positive pressure effect must again be in a

volume change when the drug denatures the enzyme with increase in molecular volume. Drugs with which pressure has no reversing effect apparently act by a different mechanism, in some cases presumably by forming an adsorption complex with the enzyme. Mathematical analysis of drug action by Johnson, Eyring, and Williams (1942), McElroy (1943), Johnson, Eyring, and Kearns (1943) and Johnson *et al.* (1945) has made it possible to distinguish two types of inhibitors. In type I, represented by sulfanilamide, the inhibitor combines with native and denatured enzyme indiscriminately; in type II, represented by urethane, the inhibitor combines in effect with only the denatured enzyme. In type I inhibitors, increasing temperature decreases the narcotic action, and there is no reversal with increase of pressure. In type II inhibitors increasing temperature increases the narcotic action, which is usually but not always reversed by pressure.

According to McElroy (1943), who studied a series of narcotics, type I inhibitors show a partition coefficient that decreases with temperature, and type II inhibitors have partition coefficients which increase with increase in temperature. Therefore the temperature effects on narcotics and luminescence of luminous bacteria correspond with the old idea of narcosis depending on distribution of narcotic between medium and fatty material in the cell. Details of the action of various substances must be sought in the original papers.

Narcotics and Anesthetics. It must be emphasized that the word narcotic is a general term and that some narcotics (barbiturates, chlorotone, chloral hydrate, trional) are type I inhibitors while others (urethanes, carbamates, alcohols, ether, procaine, benzamid, salicylamid, and monacetin) are type II inhibitors, as explained above.

Application of absolute reaction rate theory to narcotics and combinations of narcotics and sulfa drugs will be found exhaustively treated in the papers of Johnson and Williams (1942), Johnson, Eyring, and Kearns (1943) McElroy (1943, 44), and Johnson *et al.* (1945), as well as in review papers.

In addition to the early observations and the general treatment of these substances in relation to luminescence outlined above, a number of workers have studied their effect on respiration (oxygen consumption) and other activities of luminous bacteria. Measurements have been made by Taylor (1934, 36) on a series of alcohols, carbamates, and urethanes. Small concentrations frequently stimulate both luminescence and oxygen consumption, while higher concentrations reversibly inhibit the two processes. The concentration threshold for each is different and higher in the case of oxygen consumption, so that concentrations sufficient to inhibit luminescence may actually

stimulate respiration. Stimulation is greatest in absence of sufficient substrate. The effectiveness of an homologous series was found to be related to their lipoid solubility. Taylor (1936) also analyzed the curves relating concentration of ethyl urethane and per cent inhibition of luminescence and respiration, finding a complex relation possibly indicating competition between narcotic and oxygen for possession of the catalytic surfaces. Korr (1935) found that urethane in narcotic concentrations had no effect on reduction rate or final redox potential.

Further analysis of ethyl urethane effects have been made by van Schouwenburg (1938), who obtained results identical with those of Taylor and also studied combinations of urethane and cyanide, which led to the belief that urethane acted directly on luciferase, a conclusion confirmed (Johnson, van Schouwenburg, and van der Burg, 1939) by the study of the flash of light from bacteria after anaerobiosis. Schoepfle (1941) has analyzed the action of veronal, which affects luciferase, on the equation relating the light intensity of the flash and time.

The action of narcotics on oxidation of glucose, with especial reference to concentrations where respiration is stimulated and luminescence greatly inhibited, has been studied by McElroy (1944). He attributed the increased respiration to rapid breakdown of material assimilated during growth, i.e., a stimulation of the endogenous respiration.

Sulfa Drugs. Probably the first study of bacterial luminescence inhibition by these compounds, so important in therapy, was made by Johnson and Moore (1941), followed by Eyring and McGee (1942), Johnson, Eyring, and Williams (1942), and Johnson, Eyring *et al.* (1945). The lack of pressure reversal of sulfanilamide inhibition was first noted by Johnson, Brown, and Marsland (1942), and the subsequent investigations have definitely placed sulfanilamide in the group I inhibitors. The combined action of sulfanilamide (type I) and urethane (type II) on inhibition of luminescence has been treated by Johnson, Eyring, and Kearns (1943), and equations have been developed to explain the experimental results.

Para aminobenzoic acid (PAB) is also a type I inhibitor, and Johnson (1942) has suggested that its action is fundamentally like a narcotic. Since Woods designated PAB as an essential metabolite and an antisulfonamide by virtue of competition for the same locus or enzyme in the organism, there has been considerable discussion of its action on luminescence. McElroy (1944) compared the growth of *Neurospora* from Tatum and Beadle's data and the inhibition of bacterial luminescence, both as a function of concentration. There was a simi-

larity which led him to believe that the sulfanilamide was acting on a similar group in the enzymes concerned. However, Johnson, Eyring *et al.* (1945) find that in low concentration PAB antagonizes sulfanilamide inhibition of growth of various species of luminous bacteria but has no effect on luminescence in the same concentrations, or an inhibitory effect only in higher concentrations. If luminescence has been partly inhibited by sulfanilamide, adding PAB has no effect or causes a slight inhibition depending on the concentration. The conclusion was that two different, though possibly related enzyme systems, limit growth and luminescence of these bacteria.

Quinones. The author (1929) noticed that quinone, *p*-xyloquinone, 1,2-naphthoquinone, 2Cl-1,4-naphthoquinone, and 1,4-naphthoquinone dimmed the light of bacteria and were all toxic irrespective of their position in the redox scale. It was pointed out that a compound like quinone could act by rapidly oxidizing luciferin. However, since 1,4-naphthoquinone dimmed the light although its redox potential is less than that of *Cypridina* oxidized luciferin, it seemed unlikely that the dimming was due to oxidation of bacterial luciferin. The antibacterial effect of these compounds has also been emphasized by Rake and collaborators and by Kavanagh (1947). The interest of naphthoquinones for bioluminescence is considerable, as luciferin has been considered to be a 1,4-naphthohydroquinone with a $\text{CO-CH}_2\text{OH}$ side chain in the 2 position. However, Johnson, Rexford, and Harvey (1949) found that this compound dims the light of *Photobacterium phosphoreum*. Because of the possible similarity to luciferin structure, Spruit and Schuiling (1945) have made a special study of the effect of six 1,4-naphthoquinones and substitution products as well as related compounds, finding in all cases a marked inhibition of luminescence in dilute solution. The percentage inhibition of respiration was always less than the luminescence reduction. Actual concentrations depend on the number of bacteria present. They explained the result of luminescence inhibition as due to oxidation of luciferin whose redox potential was deduced to be -0.050 volt²² as a consequence of the behavior of the above-mentioned naphthoquinones and some dyes which also inhibited the luminescence. Since addition of KCN eliminated the inhibition of the naphthoquinones, Spruit and Schuiling believed the naphthohydroquinones were dehydrogenated again with the aid of a cyanide sensitive catalyst.

This interpretation is not confirmed by McElroy and Kipnis (1947), who explain the effect by enzyme inhibition. They investigated the action of 2-methyl-1,4-naphthoquinone on luminescence of

²² The value for *Cypridina* luciferin found by Anderson (1936) was $+0.270$ volt.

Achromobacter fischeri in relation to concentration, temperature, pH, number of bacteria, and glucose concentration. Decrease in pH below neutrality, decrease in temperature, disappearance of glucose from the medium, and low bacterial density increase the inhibitory effect. This naphthoquinone turned out to be a type I inhibitor, but the action is complex, and a change in the enzyme inhibited occurs at 26° as time goes on. Possible sites of attack are luciferase and a link in the main respiratory pathway. The temperature curve for naphthoquinone action is reproduced as Fig. 22.

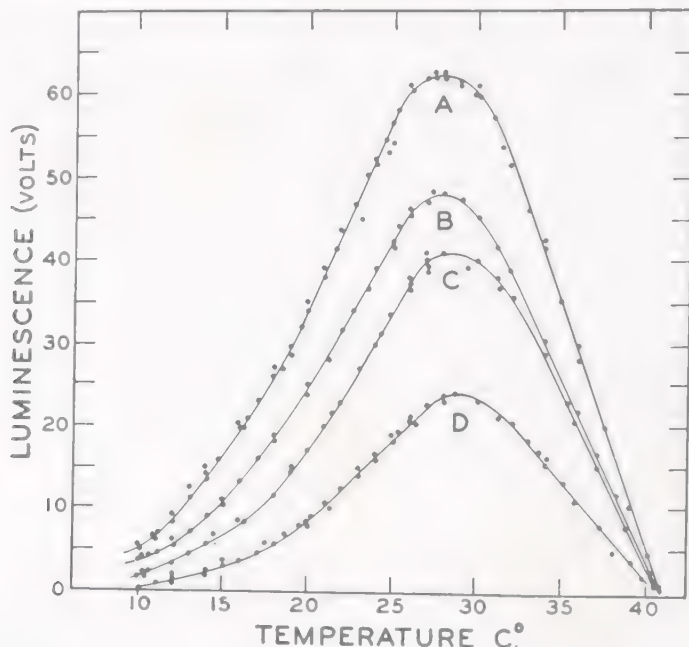


FIG. 22. The relationship between temperature and light intensity in arbitrary units of *Achromobacter fischeri*. A is the control, and B, C, D equal 2.9, 4.3, and 7.6×10^{-6} molar concentration of naphthoquinone, respectively. After McElroy and Kipnis.

Antibiotics. The effect on luminescence of natural antibiotics like penicillin is of special interest because of the possible use of luminous bacteria in bioassay of the material. Early in World War II, F. H. Johnson²⁴ tested luminous bacteria with penicillin and found no effect of the pure material on luminescence, although crude preparations often contain impurities affecting the luminescence. Rake, McKee, and Jones (1942) found that aspergillie acid from *Aspergillus flavus*, clavacin from *A. clavatus*, and actinomycin A from *Actinomyces antibioticus* in high dilution all decreased the light of bacteria, but pure penicillin did not. The bacterial test for aspergillie acid could be used (Jones, Rake, and Hamre, 1943) in bioassay.

²⁴ Unpublished observations.

Later Rake, Jones, and McKee (1943) studied a large number of antibiotics and compared their antiluminescent activity with their antibacterial action on *Streptococcus pyogenes*. Of the pure substances tolu-*p*-quinone, gliotoxin and pyocyanin, lauryl sulfate and phenol were all more effective than sulfanilamide in decreasing luminescence. The inhibitory action of clavacin was measured in a special luminometer by Griner, Tytell, and Kersten (1945). Kavanagh (1947) has also tested a large number (27) of antibacterial substances for antiluminescent activity, finding no common group to which the activity could be attributed. It was noted that the antiluminescent activity did not always parallel the antibacterial activity. The penicillins were inactive in antiluminescent tests but affected growing cultures of *Photobacterium fischeri*. Streptomycin prevented luminescence only after 24 hours of incubation.

Alkaloids and Glucosides. The inactivity of saponin in affecting luminescence of bacteria was noted by Lehmann (1889) and Harvey (1915). In 1% solution Lehmann found morphine, strychnine, and caffeine to be without effect but quinine had an inhibitory action. A very detailed study of quinine has recently been made by Johnson and Schneyer (1944), using *Achromobacter fischeri* and *Photobacterium phosphoreum*. The luminescence was measured in relation to concentration, temperature, and pressure and in combination with varying amounts of sulfanilamide. The quinine inhibition was independent of bacterial concentration and quinine behaved like a type II inhibitor, increasing with rising temperature and reversing with hydrostatic pressure. There was a mutual antagonism of quinine and sulfanilamide which might be connected with chemical combination of the two.

Colchicine in low concentration was found by Obaton (1939) to accelerate the development of luminous bacteria, as evidenced by the more rapid appearance of light during growth, with greater density of bacteria.

The unusual behavior of alpha-methylglucoside, studied by Johnson (1937) and Johnson and Anderson (1938) has already been considered.

Hormones. Substances of such varied composition cannot be expected to act in a similar manner, but their unusual physiological effect on higher animals might be connected with enzyme-inhibiting activities in bacterial cells. A study of some ten hormones, four of them (epinephrine, insulin, thyroxine, and theelin) in crystalline form showed no effect on luminescence of *Vibrio phosphorescens*, except with adrenaline, which always decreased the intensity. Theelin, epinephrine, pitocin and pitressin appeared to increase specifically the oxygen consumption, while hormone extracts of thyroid, parathyroid,

liver, and adrenal cortex and insulin increased respiration as does the nutrient, peptone. Methylene blue also markedly increased the oxygen consumption and Taylor suggested that epinephrine action on respiration was similar to that of methylene blue considered in the next section.

Dyes. Measurement of luminescence intensity after addition of dyes has not usually been made, because of the necessity to correct for dye absorption, but a number of workers have studied the change in respiration. An early observation is due to de Coulon (1916), who noticed that if methylene blue was added to a bouillon culture of *Pseudomonas luminescens* and the culture allowed to stand undisturbed, the luminescence would last a longer time, nearly in proportion to the amount of dye added. He considered that time was necessary to "remove oxygen" from methylene blue and found that when cyanide plus methylene blue was used the time was prolonged still more, because KCN "opposed the respiration" and all the oxygen was used in luminescence.

The action of methylene blue depends on the concentration. Taylor (1932) used 0.003 to 0.005 mg per cubic centimeter with *Vibrio phosphorescens* and found a marked increase in oxygen consumption, and Spruit and Schuiling also observed increased respiration of *Photobacterium phosphoreum* with both methylene blue and pyocyanine. Pyocyanine in greater concentration reduced the respiration (50% at 1.3×10^{-4} millimol) while methylene blue did not in the concentrations tested. Luminescence intensity was always reduced. More experiments should be carried out with a considerable range of concentrations, in order to compare dye effects on luminous bacteria with action on other cells. In presence of nutrient, the stimulating effect of methylene blue on respiration is much less, and it is possible that ratio of number of bacteria to methylene blue concentration is an important factor in its action.

Dinitrophenols. The recent designation of the dinitro- and dihalo-phenols as specific inhibitors of phosphorylating systems and their ability to increase the respiration of fertilized sea-urchin eggs while blocking cell division has made their action on luminescence of special interest. Shoup and Kimler (1934) first studied 2,4-dinitrophenol (DNP) action on *Achromobacter fischeri*. There was no evidence of luminescence stimulation in any concentration and light emission was not diminished until the oxygen consumption had been decreased about 55%. This decrease occurred with about 20 mg per liter of the DNP. Low concentrations (2 mg per liter) more than double the normal oxygen consumption, but the stimulating effect is only tempo-

rary. Schoepfle (1941) also found no increase in light intensity with *A. fischeri* in any concentration but a reduction of the flash intensity above 5 mg per liter without affecting constants k_2 and k_1 in the equation for flash kinetics.

In his study of glucose assimilation of *A. fischeri*, McElroy (1944) found that glucose greatly affected the behavior toward DNP. There was little stimulation if glucose was present, but DNP caused a decrease in respiration earlier than the decrease which occurs when glucose is exhausted, i.e., it affects the per cent oxidation of glucose. The luminescence intensity drops sharply at this point also. McElroy concluded that DNP affected the utilization of some intermediate produce in glucose oxidation. It is not yet known whether DNP belongs to the type I or type II inhibitors.

Cyanide. Probably no substance has been as important in the study of bacterial luminous mechanisms as cyanide. The author noticed that bacterial light was reduced somewhat in intensity by critical concentrations of cyanide in 1915 although the results were not published until 1923 when a comparison was made of KCN effects on ordinary luminous bacteria and the symbiotic luminous bacteria from the fish, *Photoblepharon*. Both types behaved in the same way. De Coulon (1916) found that *Pseudomonas luminescens* suspensions containing KCN took ten times as long to reduce methylene blue and a correspondingly long time to use up the oxygen dissolved in the medium.

This inhibitory effect on respiration is characteristic of most cells and has been measured by Harvey (1925), Taylor (1932, 34), Eymers and van Schouwenburg (1937), van Schouwenburg (1938), Claren (1938), and others. The cyanide undoubtedly acts on the heme oxidative catalysts while the luciferase is unaffected.²⁰ Evidence for the competition of the heme system and the luciferase system for oxygen has already been given in the section on luminescence and cell respiration. Competition leads to the unusual situation that inhibition of luminescence requires more cyanide at low than at high concentration of oxygen. Taylor (1934) observed a stimulating effect on luminescence and respiration by small amounts of cyanide, while Korr (1935) found the rate of potential change and final potential attained in bacterial suspensions to be unaffected by cyanide in non-lethal concentrations.

Carbon Monoxide. Another substance of special interest because of the inhibiting effect of light on its combination with heme com-

²⁰ Van Schouwenburg (1938) believed that cyanide reacts with luciferin, a conclusion demonstrated by Giese and Chase (1940) for *Cypridina luciferin*.

pounds is CO. The gas was considered neutral and non-toxic in action by Lehmann (1889), but de Coulon (1916) and Shoup (1933) noticed an inhibiting effect on respiration, and Shoup found an inhibiting effect on luminescence of bacteria (species not mentioned) only when the respiration had been reduced 55%. However, Claren (1938) reported no effect of 5 to 95% CO-O₂ mixtures on oxygen consumption of *Micrococcus cyanophos* in the dark.

Different strains of bacteria appear to react differently. Van Schouwenburg and van der Burg (1940) made an extended study of CO and *Photobacterium phosphoreum*. They found that the respiration was always inhibited, whereas luminescence increased under low CO-O₂ ratios, and decreased with high CO-O₂ ratios. Inhibition of respiration was increased if the CO-O₂ mixture was diluted with nitrogen, but there was no effect on the light intensity. Illumination, if sufficiently high, was found to counteract the CO effect on respiration and also on luminescence, whether the latter is stimulated with low CO or inhibited with high CO concentration.

An analysis of the action of CO indicates that luminescence effects are secondary, due to combination of CO with heme enzymes. According to the scheme for light emission of Johnson, van Schouwenburg, and van der Burg (p. 44), if the heme systems are inhibited, there will be an accumulation of reduced products XH₂ and LH₂ in the cell and light intensity will increase with accumulation of LH₂ (luciferin). This occurs in low CO-O₂ ratios. After still further inhibition of the heme respiration with greater CO concentration, the reducing condition become so great that autolytic decomposition of A to B occurs with decrease in luciferase and hence reduction of luminescence.

In later work, Shoup (1941), using another bacterium, *Photobacterium fischeri*, found no stimulation of luminescence with low, and relatively little reduction of luminescence with high concentrations of CO. The oxygen consumption of *P. fischeri* was also less affected than *P. phosphoreum* used by the Dutch workers. Illumination also, unless very intense, had little effect on the action of CO.

Azide. The effect of this respiratory inhibitor on *Achromobacter fischeri* has been studied by Giese (1945), who found luminescence somewhat more sensitive than respiration. Both are reversibly decreased with occasional respiratory stimulation in a low concentration of azide. Growth is also definitely inhibited. Endogenous respiration is less affected than the exogenous, the latter depending on the substrate supplied. The least effect was found with glycerol, a greater action with peptone, succinate, pyruvate, and glucose. Since the flash

after anaerobiosis was (to the eye) unaffected by azide. Giese concluded that some other enzyme system than luciferase must be inhibited.

Arsenite. Korr (1935) found that both luminescence and respiration of bacteria are greatly inhibited by 0.003*m* arsenious oxide. The reducing intensity of the bacteria was also impaired, as indicated by the fact that their redox potential only fell to -0.16 volt under anoxic conditions whereas the normal value is -0.214 volt.

Iodoacetate. According to Korr (1935) this compound impairs both respiration and luminescence of luminous bacteria and likewise prevents the attaining of the low normal anaerobic redox potential. Bromoacetate is about half as effective, and chloroacetate has practically no action.

Fluoride. Korr's (1935) results indicate that fluoride, a salt toxic to many cells, had no effect on respiration, luminescence or the anaerobic redox potential of luminous bacteria, even in high concentration (0.1*m*).

Pyrophosphate. This salt is thought to affect certain dehydrogenases. The respiration of yeast is unaffected and Korr (1935) found no effect on respiration or luminescence of luminous bacteria, although the time to reach the final redox potential (-0.214 volt at $\text{pH} = 7.6$) characteristic of anaerobic suspensions of luminous bacteria was somewhat prolonged.

Heavy Metals. As in the case of other bacteria, heavy metals are toxic to luminous bacteria in low concentration. Even the metal itself placed in cultures, will prevent growth and luminescence within a certain surrounding area. One of the earliest studies of metal effects on luminous bacteria was made by Dewar (1910). He noticed that small pieces of various metals placed in a culture dish containing the bacteria would prevent their luminescence. Unfortunately the dish was a tin plate, and electrolytic action with two dissimilar metals in a saline solution may have been involved. Unlike the heavy metals, Dewar found sulfur "actually to stimulate the bacteria which cluster brilliantly round it on the culture, leaving the rest of the plate relatively dark." The platinum group, gold, tantalum, cadmium, magnesium, tin, graphite, and selenium had no effect; bismuth, thallium, lead, nickel, german silver, hard brass, and aluminum bronze were slightly toxic, while cobalt, silver, mercury,²⁶ antimony, arsenic, and phosphorbronze were very poisonous.

²⁶ A short exposure to mercury is harmless. According to a private communication from Dr. F. H. Johnson, mercury, if free of impurities, can be shaken with luminous bacterial suspensions without affecting the light.

Metal salt studies have been made by Bukatsch (1936) who noticed the stimulating effect of Cu, Zn, Fe, Mn, and Al salts in low concentration and the toxic action in higher concentration. The ability of Ca and Sr to counteract the effect has already been mentioned.

Johnson, Carver, and Harryman (1942) have also studied the action of metal salts and the metals themselves, using the auxanographic method. They obtained beautiful color photographs of the results. Metallic copper, cobalt, cadmium, and arsenic placed in growing cultures of *Ph. phosphoreum* and *A. fischeri* were definitely inhibitory; zinc, lead, and nickel less so; manganese, magnesium, and bismuth to a doubtful extent; gold and aluminium neutral.

One of the most interesting effects with metal salts, observed also by Taylor (1934, 36) and van Schouwenburg (1938) in the action of certain drugs on luminous bacteria, was a stimulation of luminescence in very low concentrations. CuCl_2 , CoCl_2 , MnCl_2 , and NiCl_2 exhibited this effect particularly well. The zone of inhibition in high concentrations was separated from the area of normal growth by a zone of excess brightness. Johnson *et al.* have suggested that stimulation of luminescence by heavy metals and various other substances may be explained by competition between luciferase and some other enzyme systems in the bacteria for a compound involved in light production, analogous to the stimulation of light emission by KCN at low oxygen tensions.

Among heavy metals, mercuric chloride has gained the general reputation of most effective antiseptic. A special study has been made of its action on *Achromobacter fischeri*, suspended in PN²¹ by Houck (1942). The number of bacteria in a suspension proved to be important in that a direct relation exists between concentration of HgCl_2 and concentration of cells for 50% inhibition of luminescence. This relation is not so clear-cut for inhibition of oxygen consumption. Decrease in light intensity after adding HgCl_2 is very rapid but with dilute concentrations ($10^{-6}M$ with 4×10^8 bacteria per cc), where the effect can be measured, there is a logarithmic decrease in light intensity as time goes on. The per cent inhibition by HgCl_2 is greater at pH 5.3 than at pH 7.3 and much less at low temperatures ($3-6^\circ\text{C}$), which completely protect suspensions from an amount of HgCl_2 that would reduce the luminescence 50% at 25°C . The HgCl_2 inhibition cannot be reversed by diluting or centrifuging the bacteria and resuspending in mercury free media but addition of glutathione, peptone, and H₂S will partially restore the light.

²¹ See section on culture media for the composition of PN.

EFFECT OF HEAVY WATER

Deuterium oxide (D_2O) reduces both the luminescence and oxygen consumption of a marine luminous bacterium but rather large amounts are necessary (Harvey and Taylor, 1934). The effect on oxygen consumption is shown in Fig. 23. The luminescence is markedly decreased in all concentrations except 36% D_2O where the effect is slight. With a fresh water form, *Vibrio phosphorescens*, the decrease in oxygen consumption is only about half as great as in the case of the salt water species, with the luminescence little affected.

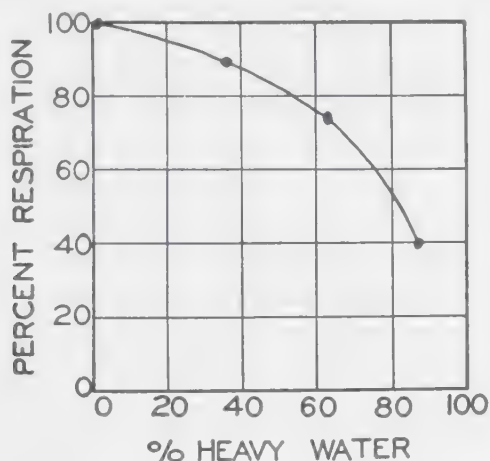


FIG. 23. The per cent reduction of oxygen consumption of marine luminous bacteria as a function of the heavy water concentration of the medium. After Harvey and Taylor.

Zirpolo (1938) found that exposure to 99.6% heavy water affected the subsequent growth of *Bacillus Pierantonii*, slowing the development and decreasing the light intensity. After two days in D_2O the bacteria would grow but not luminesce.

INTENSITY OF LIGHT EMISSION

Many early workers demonstrated that bacterial light was strong enough, if the exposure was sufficient, to affect a photographic plate (Dubois, 1886, 1900, 01; Fischer, 1888; Barnard, 1902; Nadson 1903; Molisch, 1903) and cause heliotropism of plants (Molisch, 1902; Nadson, 1903). Molisch (1904) and Richter (1906) observed no visible chlorophyll formation in etiolated seedlings, but Issatschenko (1903, 07) found traces of chlorophyll with the spectroscope. The negative visual findings are due to low light intensity as bacterial light contains wave lengths which can cause chlorophyll formation. In recent years many beautiful photographs of infected fish such as that in Fig. 1 have been

made. Even colored photographs of colonies on agar present no difficulty, as evidenced by the auxanographic studies of heavy metals of Johnson, Carver, and Harryman (1942).

Probably the first measurement of bacterial light intensity was made by Lode (1904, 06) who found for bacterial colonies about 0.7×10^{-10} international candle per square millimeter of colony, while Friedberger and Doepner's (1907) value was ten times this. Lode calculated that the dome of St. Peter's at Rome, if covered with luminous bacteria, would give little more light than one common candle. According to Steche (1909) the large luminous organ of the fish *Photoblepharon palpebratus* containing luminous bacteria is equivalent in brightness to a completely reflecting surface illuminated with 0.0024 meter-candle. The author (1925) observed a brightness of 23 to 144 microlamberts for well-aerated *Bacillus phosphorescens* suspensions in a vessel 2.7 cm thick. After correcting for the absorption of light by other bacteria, a single luminous bacterium had an intensity of 1.9×10^{-14} candle.

Johnson, Carver, and Harryman's (1942) measurements of colonies of *Photobacterium phosphoreum* on agar at optimum temperature ranged as high as 40 microlamberts and for *Achromobacter fischeri* as high as 34 microlamberts. The intensity is such that when painted on walls the bacterial growth could serve as a marker in the dark and suggestions have been made for such use of bacterial colonies in war blackouts.

Most of the early bacterial light measurements were obtained by eye comparison of the bacteria with surfaces whose brightness was known. Rapid comparison of a series of tubes can be made with such devices as those described by Eymers and van Schouwenburg (1937) and Johnson and Harvey (1938). With the advent of photocells and amplifiers light measurement becomes much easier, and short flashes of luminescence can be recorded. A description of various types of apparatus for this purpose will be found in the papers of Harvey and Snell (1930), Shapiro (1934), Eymers and van Schouwenburg (1936), Johnson, van Schouwenburg, and van der Burg (1939), Chance, Harvey, Johnson, and Millikan (1940), Butt and Alexander (1942), and Griner, Lytell, and Kerstein (1945).

The only direct measurements in absolute units have been made by Eymers and van Schouwenburg (1937). They found that 1 cc of a suspension of *Photobacterium phosphoreum* (2 mg dry weight)²⁸ emitted 7.18 ergs per second at 22°. At lower temperatures the emission was less—1.04 ergs per second at 9.1° and 2.5 ergs per second at

²⁸ The number of bacteria is not given.

16. Quantum emission is discussed in the section on efficiency. Suchsland (1898) has claimed that the light of bacteria is polarized, but the author has been unable to verify this, even when the bacteria are oriented by flow through a narrow tube.

SPECTRAL DISTRIBUTION

The Emission Spectrum. The first studies on the quality of bacterial light were made by Ludwig (1884), who established the continuous nature of the spectrum, a short band in the visible region extending from Fraunhofer line b into the violet. Subsequent observations by Forster (1887), Barnard (1899), Molisch (1904), and McDermott (1911) have confirmed Ludwig's description. The first photograph of a bacterial luminescence spectrum appears to have been published by Barnard (1902), and most of the subsequent work has been in spectrophotography. The photograph of Forsyth (1910) shows a bacterial spectrum extending into the ultraviolet as far as 350 m μ , but McDermott (1911) was unable to observe excitation of *p*-amino-*o*-sulfobenzoic acid, strongly fluorescent on exposure to near ultraviolet, by bacterial light. Schubert (1934) has also claimed short wave radiation to be produced but it seems fairly certain that no appreciable ultraviolet light is emitted.

Several older writers (Fischer, 1888; Molisch, 1904; Dubois, 1914) have reported that the light of luminous bacteria changes in color if grown on different culture media. Light which is "silver white" on dead fish becomes "greenish" on salt-peptone-gelatin media and more yellow on salt-poor media. Such observations may be subjective, due to different conditions of the retina. They are questionable without actual knowledge of spectral energy distribution.

The first records of this type were made by Eymers and van Schouwenburg (1936, 37), who studied spectral energy curves of several species of bacteria, plotting the energy both as a function of wave length and of frequency.²⁰ The latter method of plotting was believed to bring out a few fundamental frequencies whose symmetrical broadening and combination gives rise to the complete curve. Bacterial spectrograms for *Photobacterium phosphoreum*, *Ph. splendidum*, and a fresh water *Vibrio* all showed fundamental frequencies at 18,300 cm⁻¹ and 20,400 cm⁻¹, but Eymers and van Schouwenburg could detect no difference in the spectral energy curves of *Photobacterium phos-*

²⁰ Unpublished.

²¹ Frequency is defined as the reciprocal of wave length expressed in centimeters $\nu = 1/\lambda$. To obtain actual frequencies the figures must be multiplied by the velocity of light, 3×10^{10} cm per second.

phoreum and *Ph. splendidum* and no differences connected with the age of the culture or external conditions such as temperature, salt content, hydrogen ion concentration or nutrient in the medium. De Lerna (1946) found the similar fundamental frequencies at 18,000 and 20,300 cm^{-1} for *Bacillus sepieae* and *Vibrio pierantonii*.

Takase's (1938, 40) analysis of the light of symbiotic bacteria from the deep sea fish, *Coelorrhynchus* showed fundamental frequencies at 21,200 cm^{-1} and 19,200 cm^{-1} . He claimed that on a cane sugar medium the 21,200 cm^{-1} is the more intense, while the reverse is true on a salt medium. Akabane's (1938) curve for bacteria No. 7, isolated from Macrourid fishes was symmetrical, extending from 620 to 420 $\text{m}\mu$ with a maximum at 520 $\text{m}\mu$.

Further study has indicated that fundamental frequencies common to many different chemiluminescences are unlikely to occur. In a comprehensive survey of absorption spectra and fluorescence, chemiluminescence and bioluminescence spectra, van der Burg (1943) has stressed the importance of eliminating the selective absorption by such compounds as riboflavin or cytochrome within the bacterial cell; otherwise the spectral distribution curve will be affected. When this absorption is rendered as small as possible by dilute bacterial suspensions, the curves obtained by van der Burg (1943) and Spruit-van der Burg (1950) are different from those of Eymers and van Schouwenburg. No fundamental frequencies appear and the spectra for *Photobacterium phosphoreum*, *Ph. splendidum*, and *Ph. fischeri* are slightly different in shape and have different wave lengths of maximum emission. The curves of the three species of bacteria and the fungus, *Armillaria mellea*, corrected for absorption, are reproduced as Fig. 24.

In van der Burg's study of fluorescence, the mirror symmetry of the excitation (absorption) spectrum and fluorescence spectrum of some substances (fluoresceine or rhodamine 6G) is referred to and an attempt made to find mirror symmetry in the action spectrum for inhibition of luminescence of bacteria (obtained by van der Kerk, 1942) and the emission spectrum of the bacteria corrected for absorption. The two spectra plotted on a frequency scale are reproduced in Fig. 25. It will be observed that a partial symmetry does exist, which led van der Burg to the suggestion that bacterial "bioluminescence might be a chemofluorescence as a consequence of transfer of reaction energy to a pigment adsorbed on the luciferase which at the same time acts as a sensitizer in the photochemical inactivation." This paper also contains a detailed analysis of the chemiluminescence of phthalhydrazid derivatives and of dimethyldiacridinium nitrate in relation to absorption and fluorescence.

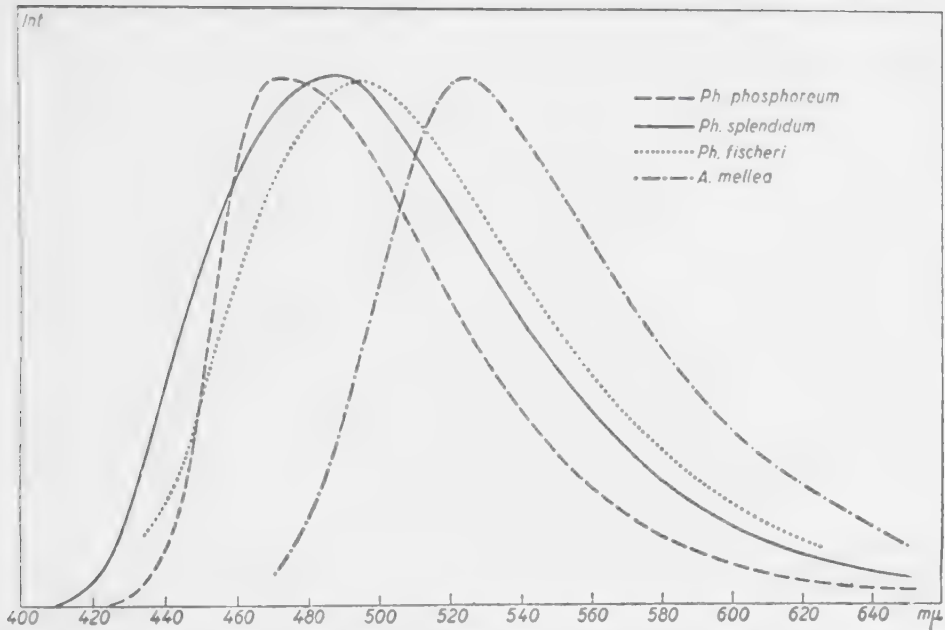


FIG. 24. The emission spectra of three species of *Photobacterium* and a fungus, *Armillaria mellea*. After Spruit-van der Burg, in *Biochimica et Biophysica Acta*, by courtesy of Elsevier Publishing Co. Inc.

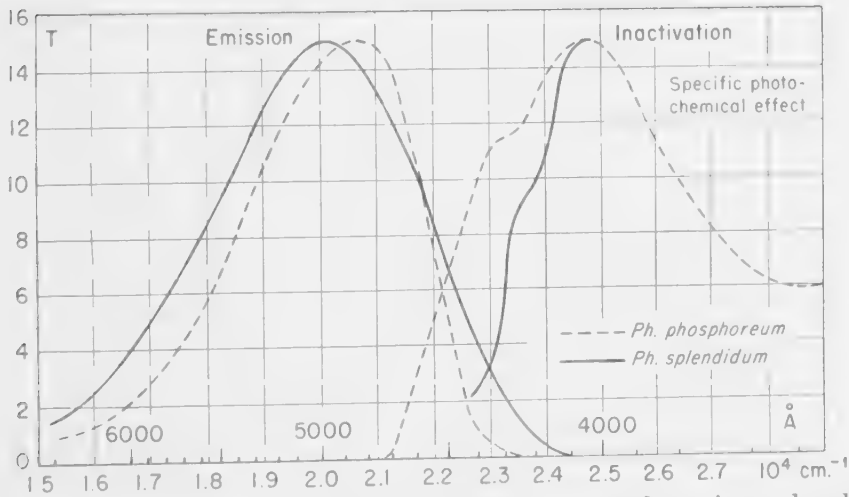


FIG. 25. Emission and inactivation curves for *Photobacterium phosphoreum* (dotted line) and *Ph. splendidum* (continuous line) showing mirror symmetry. Light intensity (left vertical) and suppression of luminescence (right vertical) are plotted versus wave length in angstroms and frequency in cm^{-1} (horizontal). After van der Burg.

Penetrating Radiation. Discovery of X rays in 1895 stimulated the search for penetrating radiation, and Dubois (1896, 1901) described an experiment with liquid cultures of photobacteria placed under a photographic plate wrapped in three layers of black paper with a coin interposed between the layers. After 24 hours' exposure in a dark room, the plate on development showed an effect coming from the bacteria, which was intercepted by the coin.

The existence of such radiation from bacteria has been denied by Suchsland (1898), Schurig (1901), and Molisch (1904). The experiments of Molisch are of greatest interest, for they are very carefully controlled and show without a doubt that black paper or metal sheet will allow no rays from these organisms to pass that will affect a photographic plate, even after several days' exposure. The *visible* light of luminous bacteria will affect the plate after one second's exposure. Moreover, Molisch has pointed out the errors of those who claim to have found penetrating radiation from luminous forms. Certain kinds of cardboard, especially yellow varieties, or wood, will give off vapors that affect the photographic plate. The action is especially marked with damp cardboard at a temperature of 25 to 35°C. A piece of old dry section of beech or oak trunk, placed on a photographic plate for 15 hours in a totally dark place, will register a beautiful picture of the annual rings of growth, medullary rays, junction of bark and wood, etc. Early workers, particularly Russell (1897), had previously found that many bodies, both metals and substances of organic origin (resins, wood, paper, etc.), placed in contact with photographic plates, would affect them, and concluded that vapors and not rays were the active agents. Frankland (1898) also noted vapors coming from the non-luminous species, *Bacillus proteus vulgaris*, and *B. coli communis*, which affected a photographic plate laid directly over the colonies in an open petri dish. There was no effect if the glass cover of the petri dish was between plate and bacteria. The "Russell effect" is in reality a chemical action of reducing or of other gases on the plate. Hydrogen peroxide, widely produced in many reactions, is extremely active and usually responsible for the effect. Keenan (1926) has reviewed the subject and literature, which is unusually extensive.

Biological Effects. Apart from heliotropic bending and development of chlorophyll in seedlings, it has been held by Zirpolo (1931) that luminous bacteria can affect the germination of seeds and by Soru and Brauner (1932) that a culture of luminous bacteria in a quartz tube will greatly stimulate cell division in rabbit bone marrow. There was no effect in a glass tube. Such results in the 1930's were attributed to mitogenetic rays, but the reality of this type of radiation has been

seriously doubted, and the above experiments with luminous bacteria should be repeated under carefully controlled conditions with statistical analysis of the results. Although not a biological effect, it should be recorded that Zirpolo has claimed that *Bacillus pierantonii* would disturb the Liesegang ring formation, which results from mutual precipitation of $K_2Cr_2O_7$ and $AgNO_3$.

EFFICIENCY OF LIGHT PRODUCTION

By the word efficiency a number of quite different quantities may be referred to. Efficiency may mean the ratio of the energy in the visible region of the spectrum of a light to the energy in the total spec-

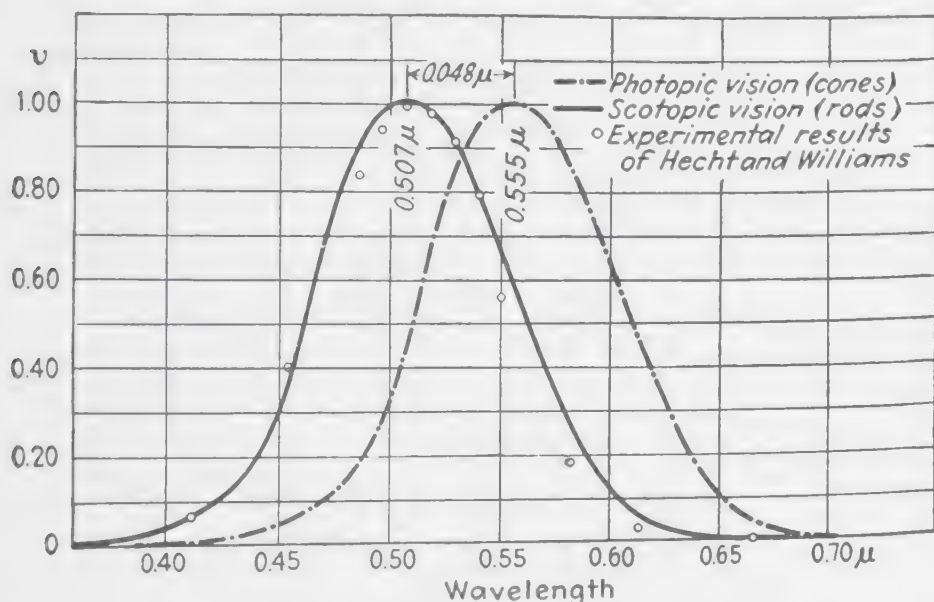


FIG. 26. Visibility of radiation curves for an average light adapted (broken line) and dark adapted eye (solid line). The relative visibility (vertical) is plotted against wave lengths (horizontal) containing equal energy. Note shift of the maximum toward the blue end of the spectrum during dark adaptation. By permission from *THE SCIENTIFIC BASIS OF ENGINEERING* by P. Moon, Copyright, 1936. McGraw-Hill Book Company, Inc.

trum, including infrared and ultraviolet wave lengths. Since luminous bacteria and luminous organisms emit only in the visible region, their efficiency on this basis is 100%.

If account is taken of the fact that not all visible wave lengths are equally well seen by the human eye, another value, luminous efficiency, is obtained. The energies of each wave length are multiplied by the visibility of that wave length and the sum of these values, the luminous flux, is then divided by the total radiant energy, the radiant flux. The visibility is given by the visibility of radiation curve, a sub-

jective expression of the effective action of different wave lengths in stimulating the human retina. For the average light adapted eye (photopic vision), the maximum of visibility is at $555\text{ m}\mu$ and for the dark adapted eye (scotopic vision) at $507\text{ m}\mu$. Visibility approaches zero at the violet and red ends of the spectrum as shown in Fig. 26. Hence, if the emission maximum of luminous organisms happens to be near the yellow-green of $555\text{ m}\mu$, as in the fire fly, the luminous efficiency will be very high, around 92%, found by Coblenz (1912) for *Photuris pennsylvanica*. In the case of luminous bacteria, where the

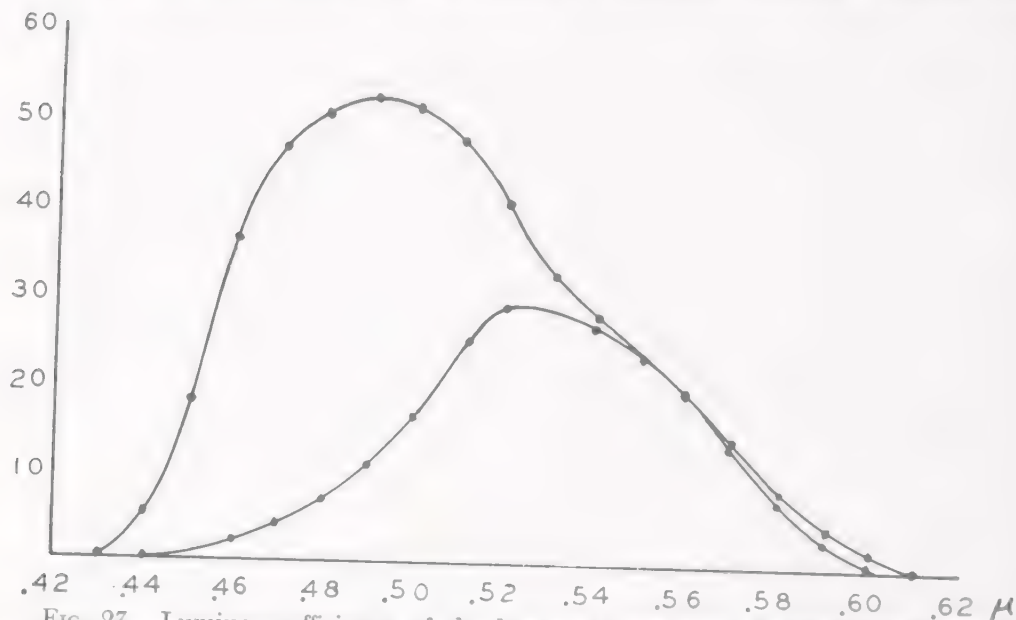


FIG. 27. Luminous efficiency of the luminous bacterium, *Photobacterium phosphoreum*, the ratio of the area under the lower curve (luminous flux) to the area under the upper curve (radiant flux), 45%. The luminous flux has been drawn by the author in a curve of Eymers and van Schouwenburg for radiant flux.

emission maximum³¹ is near $490\text{ m}\mu$ for *Photobacterium phosphoreum*, the luminous efficiency is about 45% as illustrated in Fig. 27.

Although the units used for measuring visible light, such as the candle or the lumen, are subjective units, attempts have been made to convert them to absolute energy units by finding the energy in one lumen at the wave length of maximum visibility, $555\text{ m}\mu$. This value turns out to be about 621 lumens per watt or one lumen = 0.00038 calorie, often spoken of as the "mechanical equivalent of light." For other wave lengths the value 621 lumens must be reduced a certain per

³¹ The emission maximum of about $490\text{ m}\mu$ for *Ph. phosphoreum*, obtained by Eymers and van Schouwenburg (1936) when corrected for scattering of light in the bacterial suspension shifts to near $470\text{ m}\mu$.

cent. determined from the visibility of radiation curve. Even when corrected for visibility, luminous efficiency of a bacterium is high compared to that of a candle, oil lamp, gas, or electric light, where a huge amount of energy is radiated as heat.

The high luminous efficiency of the bacteria tells nothing about the efficiency by which the chemical energy of oxidation of luciferin is converted into radiant energy, i.e., the radiational efficiency. This ratio would be the total energy in the light emitted corrected for visibility, divided by the heat of oxidation of luciferin. Unfortunately, the radiational efficiency cannot be determined, as the heat of oxidation of luciferin is unknown.

Finally there is the overall efficiency, the total energy in the light emitted corrected for visibility, divided by the energy in the food necessary to maintain the bacteria during production of luciferin and light emission. The overall efficiency regards the luminous bacteria as power plants for producing light, and as such their efficiency may be compared to practical illuminants in use today.

The energy for practical illumination originally came directly from combustion although it now comes through electric energy. The gas light, for example, involves heating carbon particles or some other material (like a Welsbach mantle) by the heat of oxidation of the hydrocarbons in the flame, and without oxygen there would be no more light than is the case with luminous bacteria. The total efficiency of a gas lamp is made up of the product of two efficiencies. The first is luminous efficiency, the ratio previously considered; the second is radiational efficiency, the energy of the total radiation divided by the energy of combustion of the gas. If the energy in the coal from which gas is produced is considered, the efficiency of the conversion of the coal to gas must also be calculated. Such a value is the overall efficiency of a gas lamp, from energy in coal to visible light. It is this value about which little is known in luminous animals.

Similar reasoning applies in the case of electric light. When an incandescent lamp lights, coal is being burned in some power house. Some energy is lost in generation of current, as only about 33% of the energy of the coal appears as electric energy at the lamp terminals. As a pound of coal in burning uses up a very definite amount of oxygen, the energy of the coal in calories can be calculated by measuring the oxygen consumed in burning. In the lamp, electric energy is converted into total radiant energy of all wave lengths, and this transformation may be very efficient in the case of a tungsten nitrogen filled lamp, about 90%. However, the most wasteful transformation is from total radiation to visible radiation. The visible radiation is only 3.6%

of the total radiation and 96.4 per cent is waste heat. The "overall" efficiency is the product of all these efficiencies and represents the per cent of energy in the coal which appears as visible light. For the best incandescent lamp it is about 1%.

In the case of luminescence, the overall efficiency can be easily determined by measuring the light emitted by a known number of bacteria and also the energy in the food consumed by the bacteria, expressing both values in calories per second. The light emitted in calories divided by food consumed in calories then gives the overall efficiency. Actually the calories in the food can best be measured by the method of indirect calorimetry, a determination of the oxygen consumed. The latter always represents a given number of calories for a particular foodstuff oxidized.

The author (1925) applied this method to a suspension of luminous bacteria, a strain of *Bacterium phosphorescens*, whose oxygen consumption was measured when fed upon 60% glycerine and 40% peptone. With such a diet each milligram of oxygen consumed should produce 3.4 gram-calories. The light measurements were made on the same suspension containing a certain number of bacteria per cubic centimeter and corrections made for the absorption of light by bacteria in front of others. The amount of light, in lumens, which each bacterium would emit in all directions, provided there were no absorption, was then calculated. As one candle emits 4π lumens, the candlepower of the smallest known light could be obtained, about 2×10^{-14} candle for a single bacterium. By converting the energy of the lumens of light of the maximum wave length (510 $m\mu$) emitted into calories, the overall efficiency of a bacterium turned out to be 0.16%. This figure tells the percentage of all the energy necessary to run a bacterium which appears as light. It does not give a true picture of the efficiency of the light-producing reaction, for much of the oxygen consumed is used by bacteria for growth and other processes which have nothing to do with luminescence.

Unfortunately it is not known what per cent of the oxygen consumed by a bacterium is used for non-light emitting oxidations, but by treating bacteria with potassium cyanide, the total oxygen consumption can be reduced to one twentieth of the previous value while the light is only slightly affected, perhaps reduced to one quarter. These experiments indicate that at most only one fifth of the oxygen is used in luminescence, and probably very much less than this. Allowing four fifths of the oxygen for the non-luminescent oxygen consumption brings the efficiency of the bacterium to nearly 1%, $0.16\% \times 5$, a figure about the same as that for overall efficiency of the ordinary in-

candescent lamp calculated from the energy of coal used in generating current.

The various efficiencies for a 40-watt tungsten vacuum lamp are: coal to electric energy, 33%; electric energy to radiant flux (radiant efficiency), 68%; luminous efficiency, 2.8%; overall efficiency, 0.63%. The gas-filled tungsten lamp might be twice as efficient and the fluorescent lamp four or five times as efficient. The only corresponding figures available for the bacteria are the luminous efficiency 45% and the overall efficiency with cyanide 0.8% and without cyanide 0.16%.

By converting the light emitted into quanta for the maximum frequency in the bacterial light of wave length ($510\text{ m}\mu$) we can calculate that one bacterium emits 1.280 quanta per second and absorbs 215,000 molecules of oxygen per second, i.e., one quantum of light appears for every 168 molecules of oxygen absorbed at about 22° . If only 20% of the oxygen is used in luminescence, 33 oxygen molecules entering the bacterium could produce one quantum.

Eymers and van Schouwenburg (1936, 37) also investigated the oxygen consumption and quantum relationships of luminous bacteria, coming to the conclusion that about 20% of the oxygen consumption is involved in the light-emitting process.³² Photobacterium phosphoreum was used and the light intensity measured in absolute units. The total oxygen consumption was corrected for that fraction involved in light emission, about 20%. Results varied with the temperature. The molecules of oxygen to produce one quantum at 9°C was 800; at 16° , 450; and at 22° , 195 molecules. These values are higher than the maximum found by the author using a less direct method. Since differences in strain of bacteria or age of the culture profoundly affect the light emitted in relation to the oxygen absorbed, a considerable variation in overall efficiency is to be expected. However, the interesting point is that luminous bacteria and incandescent lamps exhibit about the same overall efficiency.

³² Later experiments of van Schouwenburg (1938) have led to much lower estimates.

CHAPTER II

Fungi

INTRODUCTION

The light of luminous wood,¹ like that of dead fish and flesh, was known to Aristotle, and Pliny mentions a luminous fungus which Hennings (1904) considered most likely to be *Pleurotus olearius*, common on olive trees in Mediterranean countries. The relation of luminous wood to the fungus mycelium responsible for the light was not fully understood until the early nineteenth century, although there had been previous suggestions that the light was due to living organisms and Von Humboldt in 1799 believed it to be fungal in origin. He based his opinion on observations of a mine superintendent, Freyesleben, who noticed fungal growths in 1796.

Conrad Gesner, Francis Bacon, Thomas Bartolin, and Robert Boyle all observed or experimented with luminous wood, and Robert Plot in 1686 noticed the luminescence of turf. In the eighteenth century, the prize essays of Dartous de Mairan and Cohausen in 1717 and the papers of Albrecht in 1740, von Meidinger in 1777, and le Sage in 1778 contributed to the growing knowledge of this remarkable phenomenon which immediately taxed the ingenuity of philosophers for an explanation. Von Meidinger thought the light of wood was due to little animals ("Tierchen"), too small to be seen with the microscope, basing his views on Baker's discovery in 1742 that the light of the sea was due to "insects." He held that the animacules appeared in the wood only when it reached a certain degree of rottenness, and they luminesced only as long as they lived, because after drying the wood no longer produced light, and it was natural to suppose that drying killed them.

Toward the end of the eighteenth century great interest was shown in the behavior of luminous wood placed in different gases, especially oxygen, as the light was supposed to result from slow combustion. Definite knowledge of the fungal origin of luminous wood came from a study of luminous timbers used as supports in mines, due to the

¹Not to be confused with the sparkling of wood, due to collembolid insects.

careful observations of Derschau and a number of workers: Bischoff, Nees von Esenbeck, and Nöggerath (1823), and Bishoff (1823). These men described the luminous rhizomorphs and the finer strands of mycelium in the wood. Schmitz (1843) also studied the rhizomorphs, whose luminous properties were discussed in the botanies of Agardh, de Candolle, and Meyen. Their view was made certain by Heller (1853), who established beyond doubt the fungal origin of the light of luminous wood and most vegetable material, fruit, roots, etc. Despite the evidence of Heller, Hartig (1855) still held that the light originated from chemical processes in the wood, and Patouillard (1882) incorrectly believed the light of fungi to be due to luminous bacteria.

Modern experimental work begins with Fabre (1855), who made an exhaustive study of the phosphorescence of *Agaricus olearius*. Among other experiments Fabre determined that the light ceased in a vacuum, in hydrogen and CO_2 , and in water from which the air had been removed by boiling but not in oxygenated water. In pure oxygen the light was no brighter. The light was independent of moderate humidity and temperature changes and also of light, continuing night and day. He studied especially the respiration of the fungus, an inquiry inspired by Tulasne (1848), who had previously suggested that a measurement of CO_2 and heat production of luminous and non-luminous parts would be important in determining the origin of the light. Fabre found no noticeable elevation of temperature that could be measured with a thermometer, but did observe more CO_2 produced by the luminous pileus than by non-luminous parts.

All the general works on bioluminescence and botanical physiologies devote more or less space to luminous fungi, and special treatment will be found in the book of Molisch (1904, 08), the researches of Buller (1924, 34) and of Bothe (1928, 30, 31, 35), and the review articles of Klein (1928) and Wassink (1948). Popular accounts have been written by Crie (1882), Schertel (1902), Hemmings (1904), Chandler (1908), Murrill (1920), and Guyot (1928).

DISTRIBUTION OF LUMINESCENCE; CLASSIFICATION

In addition to papers dealing with the cause of the light, a large descriptive literature on various species of luminous fungi has appeared. This has been reviewed in a recent comprehensive publication of Wassink (1948) to which the reader is referred for a critical evaluation of species identity. The more important previous lists are contained in the works of Berkeley (1857), Crie (1882), Phillips (1888), von Lagerheim (1889), Zopf (1890), Ludwig (1892), Hemmings (1903), Dubois (1898), McAlpine (1901), Mangold (1910), Molisch (1912), Murrill

(1915), Buller (1924, 1934), Klein (1928), Bothe (1931), and Haneda (1939, 42 in Japanese).

Wassink listed 65 species, distributed among some 22 genera, previously reported luminescent. Of these, he regarded 42 species well established as luminescent, but because of duplication of reports, 8 might be synonyms, and 17 required further investigation to establish validity as species, leaving 17 certain luminous species. Luminescence of the remaining 23 among the 65 species listed was doubtful, particularly so in 6 of the 23.

Wassink's 17 well-known luminous species are *Armillaria mellea*, *Pleurotus olearius* (for which he regarded *Clitocybe illudens*, *Panus illudens*, *Pleurotus lampas*, *P. phosphorus*, *P. illuminans*, *P. facifer* and *P. candescens* are synonyms),² *Pleurotus japonicus*, *Panus stipticus luminescens*, *Omphalia flavida*, *Mycena illuminans*, *M. polygramma*, *M. tintinnabulum*, *M. galopus*, *M. epiptergia*, *M. sanguinolenta*, *M. dilatata*, *M. stylobates*, *M. zephira*, *M. parabolica*, *M. galericulata calopus* (for which *M. inclinata* is a synonym) and *Polyporus rhpidium*.

In the following classification of fungi, excluding the bacteria (Schizomycetes), families and orders which contain reported luminous species are printed in italics even though their light production is doubtful and further investigation may be necessary to establish their validity:

Myxomycetes

Exosporeae (Ceratiomycaceae only)

Myxogastres (or *Endosporeae*) (4 orders, 14 families, including *Didymium*)

Phycomycetes

Archimycetes (2 orders, 4 families)

Oomycetes (5 orders, 10 families)

Zygomycetes (2 orders, 10 families)

Lichens (most lichens belong in the *Ascomycetes*, a few in the *Basidiomycetes*, and are made up of combinations of algae and fungi)

Ascomycetes

Hemiascomycetes (2 orders, 5 families)

Euscomycetes (13 orders, 57 families, including *Xylaria* of the *Xylariaceae*, *Sphaeriales*)

Basidiomycetes

Heterobasidiomycetes

Tremellales or Judas ears and Jelly fungi (8 families)

Uredinales or Rusts (2 families)

Ustilaginales or Smuts (3 families)

² Even though these fungi are one species, the names applied by various authors in their study will be retained.

³ From G. W. Martin, A key to the families of fungi exclusive of the lichens. *Univ. of Iowa Studies in Natural History*, Supplement 13, 1-64, 1941.

Homobasidiomycetes

Exobasidiales (Exobasidiaceae)

Agaricales or Mushrooms and Toadstools (6 families)

Hymenogastrales or Underground puff-balls (4 families)

Phallales or Stinkhorn fungi (*Clathraceae*, including *Clathrus* or *Ilciodictyon* and *Phallaceae*, including *Kalchbrennera*)

Lycoperdales or Puff-balls (3 families)

Sclerodermatales or Puff-balls (4 families)

Nidulariales or Bird-nest fungi (2 families)

Fungi imperfecti (at least 4 orders, 11 families, life histories not well known)

Ascomycetes

The great majority of luminous fungi, whether of certain or doubtful luminescence, belong in the Basidiomycetes. However, one genus, *Xylaria* of the Ascomycetes, has been reported as having a luminous mycelium by Ludwig (1874, 84) Crie (1881, 82), and Gueguen (1907), and it is included in a list of luminous American fungi prepared by Murrill (1915). Ludwig and Gueguen studied *Xylaria hypoxylon* and Crie *Xylaria polymorpha*, both agreeing that only the mycelium is luminous and feebly so. However, Molisch (1904, 12) was unable to obtain light from pure cultures of *Xylaria hypoxylon* or *X. cookei*, and Wassink (1948) saw no light in cultures maintained in Holland. As conditions for luminescence in culture media are often quite critical and since it is known that sometimes one strain of a fungus is luminous (the American *Panus stipticus luminescens*) while another (the European *Panus stipticus non luminescens*) is not, Buller (1924) came to the conclusion that possibly two varieties of *Xylaria* existed. At any rate, the name *Xylaria* must be regarded as a possible luminous form and the Ascomycetes recognized as a group that may emit light. None of the Phycomycetes is luminous.

Basidiomycetes, Agaricales

Modern classification recognizes a large number of genera, divided among six families within the hymenomycete order, Agaricales. Of the representative genera listed below, at least 18 have been reported to be luminous, as indicated by italics in the following list:

Thelephoraceae (*Corticium*, *Peniophora*, *Stereum*, *Hymenochaete*, *Tomentella*, *Thelephora*)

Clavariaceae or Coral fungi (*Clavaria*, *Pistillaria*)

Hydnaceae or Hedge hog fungi (*Odontia*, *Radulum*, *Steccherinum*, *Sarcodon*, *Dentinum*, *Hydnum*)

Polyporaceae or Tube bearing fungi (*Polyporus*, *Dictyopanus*, *Lenzites*, *Laschia*, *Fomes*, *Poria*, *Fistulina*, *Trametes*, *Merulius*)

?*Boletaceae* or Tube-bearing fungi (?*Ceriomyces* = ?*Boletus*, *Boletinus*, *Strobilomyces*)

Agaricaceae or mushrooms and toadstools (*Panus*, *Mycena*, *Collybia*, *Lactarius*, *Helomyces*, *Cantharellus*, *Gomphidius*, *Hygrophorus*, *Hypholoma*, *Panaeolus*, *Clitopilus*, *Cortinarius*, *Pholiota*, *Amanita*, *Lepiota*, *Coprinus*, *Tricholoma*, *Agaricus*, *Armillaria*, *Pleurotus*, *Omphalia*, *Clitocybe*, *Marasmius*, *Locellina*, *Naucoria*, *Russula*)

The undoubted luminous forms are restricted to the Polyporaceae and Agaricaceae. One fungus, difficult to recognize, was called *Didymium* by Berkeley. It was described and figures anonymously in the *Gardener's Chronicle* for 1874 from St. Kitts and also reported from Jamaica. The fruiting bodies, which grow on leaves, were luminous. Wassink (1948) has considered this fungus to have been a *Mycena* or an *Omphalia*, since *Didymium* is a genus of the Myxomycetes or slime-molds, none of which is known to be luminous.

Thelephoraceae. *Corticium coeruleum* (= *Auricularia phosphorea*) of the Thelephoraceae has also been reported luminous by Tulasne (1848), Smith (1871), Cooke and Berkeley (1878), and Crie (1882), but Wassink (1948) considers the designation very doubtful.

Polyporaceae. *Polyporus rhipidium* was described by Berkeley from North America and has been reported from Australia also. The mycelium is luminous and possibly the fruiting body as well. Kobayashi (1937) also described a *Polyporus rhipidium* on dead tree trunks from the Bonin Islands. Only the pore surface was luminous, not the upper surface of the pileus and not the mycelium but the pale blue light could be seen at a distance of 2 meters. He recently found this fungus in Japan and called it *Dictyopanus pusillus* = *Polyporus rhipidium* var. *pusillus*, while Corner (1950) has suggested that a luminous form from Malaya, *Dictyopanus luminescens*, may be the same as *P. rhipidium* of the Bonin Islands. Haneda (1939) described the luminescence of *P. hanedai* from North Borneo, shown in Fig. 28 and (1942) an allied form, *P. microporus*, from Ponape. In *P. mycenoides* of New Caledonia, described by Patouillard (1889), and *P. noctilucens* of Angola, described by von Lagerheim (1889) the pileus was luminescent, but observations on the mycelium are lacking.

Smith (1872) has given a very definite account of luminous *Polyporus* (*Fomes*) *annosus*, growing on timbers of coal mines at Cardiff. Mycelium and fruiting body were both luminous and so bright that they could be seen at a distance of 20 yd. He had also seen luminous *P. sulfureus*, which had been reported luminous by Roumeguere (1882) although Buller's (1924) observations were negative for the fruiting body, as were those of Molisch and Wassink for the mycelium. However, there seems to be no doubt of the luminescence in the genus *Polyporus*.

Boletaceae. *Cerionomyces crassus* (= *Boletus impolitus*) has been mentioned as luminous, discussed by Molisch (1908) and will be found in Murrill's (1915) list, but Wassink (1948) regards the report as very doubtful.

Agaricaceae. Luminous genera of the Agaricaceae are the best known among the fungi, and experimental studies have been made on six common genera. Among the agarics, only the fruiting body, in whole or in part, or only the mycelium, or both may be luminous, depending on the species. Identification of species from a piece of lumi-

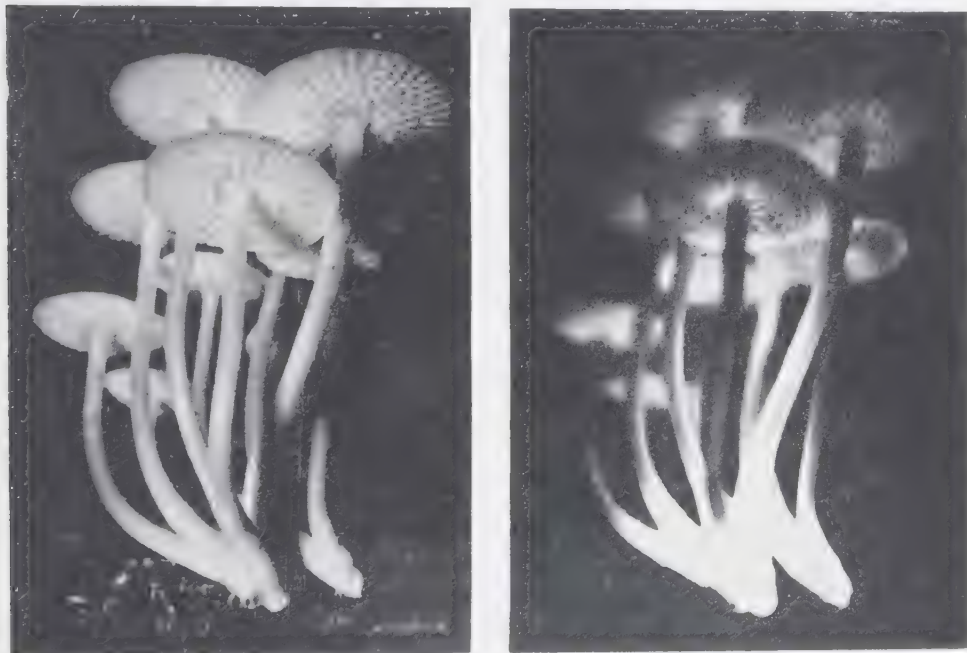


FIG. 28. *Polyporus Hanedai*, photographed by daylight (left) and its own light (right). Photos by Y. Haneda.

nous wood is practically impossible unless the fruiting body is also present, and even then there is no assurance that a luminous mycelium belongs to the particular fruiting body observed on the wood. Consequently many false reports of luminous fungi are to be found in the various lists and growth in pure culture is the only sure method of identification.

***Armillaria mellea*.** When it was first recognized that the light of wood was due to a fungus, the name *Rhizomorpha* was applied to the growth and various species were described, such as *R. subterranea*, *R. setiformis*, and *R. fragilis*. These *Rhizomorphae* are hardened masses of mycelial strands, particularly characteristic of *Armillaria mellea*, the honey colored agaric or "Hallimasch." *Armillaria* grows on old

stumps, dead trees, or buried roots and is widely distributed throughout the temperate regions of the world. The mycelium and not the fruiting body is luminous, and most persons have not suspected luminosity in this species, although Buller (1924) has reported that on some occasions a faint light may be visible in central tissue of the lower part of the stipe.

According to Molisch, Buller, and Wassink, *Armillaria mellea* is the source of practically all the light of dead wood when no obvious fruiting body is present. The records of such findings are too numerous to mention. It was studied by Tulasne (1848) as *Rhizomorpha subterranea* and by Ludwig (1874) and probably first grown in culture by Brefeld (1877). Many others have since grown it in pure culture: Kutscher (1897), Molisch (1904), Nobecourt (1926), Guyot (1927), Bothe (1928), Bose (1935), Hamada (1940), and Wassink (1948). The spores are not luminous and Wassink observed no luminescence even when germination was visible with the naked eye, but five days later (at 23°) when rhizomorphs appeared, luminescence of the mycelium began and might last for months or years. Murrill (1915) has observed that rhizomorphs must be covered with active hyphae in order to luminesce and Nobecourt (1926) has emphasized the simultaneous appearance of luminescence and browning, both of which depend on oxygen. Rhizomorphs forming a cuticle and entering the resting period lose their luminosity. They become brown or almost black after long contact with air. Another characteristic is the separation of drops of brownish non-luminous fluid, a process known as guttation, from the mass of fungal growth.

Mycelium X. Another fungus in which the mycelium is luminous is the famous *Mycelium X*, whose fruiting body has never been seen. It was isolated by Molisch (1904) in 1900 from oak bark obtained in the forest of Kuchelbad near Prague. Although a tree-growing fungus, the mycelium is quite different from *Armillaria mellea*. It can be maintained in large flasks for years with a bright and long-lasting light. Bothe (1928) has made an exhaustive study of *Mycelium X* on various culture media, without succeeding in stimulating the formation of a fruiting body. He thought it might be a species of *Mycena* (1931) but was certain it could not be *Mycena tintinnabulum*, which he (1930) had previously isolated in pure culture.

Pleurotus (Clitocybe) olearius and *Clitocybe illudens*. These two fungi are regarded by some as identical.¹ The first is a well-known

¹C. H. Kauffman (*Papers Mich. Acad. Sci. Arts Letters*, 8, 153-214, 1927) has considered *C. illudens* a different species from *P. olearius*, chiefly distinguished by its brighter orange-red color and smaller globose spores.

mushroom of Europe in which both mycelium and fruiting body are luminous, the agaric d'olivier, common at the base of olive trees. The olive agaric has been extensively studied by Raffenu-Delile (1833), Tulasne (1848), and Fabre (1855), whose experimental approach has already been mentioned. Later papers by Krukenberg (1887), Archangeli (1889), and many short notices of its luminescence have appeared (Goutaland, 1936; Josserand, 1937; Buchet, 1937). Bothé (1930), Obaton (1943), and Wassink (1948) have grown it in pure culture. In this species the gills are always luminous and usually the whole pileus but the spores are not luminous.

The second fungus, *Clitocybe illudens*, is known in the United States as "Jack-my-lantern" because of its luminosity. It has been described as a rich saffron yellow in color, non-edible, sometimes 5 in. across the pileus. An early description of its luminescence is by Atkinson (1889), who observed light coming from the hymenium and adjacent hymenophore but not from the stipe or fleshy part of the pileus. Murrill (1915) thought the mycelium must be luminous, as he had found *Clitocybe illudens* fruiting bodies on a piece of wood, which was also luminous. Buller (1924) repeated the statement but held that Murrill's wood might have been infected with *Armillaria mellea* mycelium, and Hanna (1938), by growing *C. illudens* in pure culture, has found the mycelium to be non-luminous but the fruiting bodies to emit light. There are probably both luminous and non-luminous strains, as the author has received a strain from Dr. R. Macrae in which the mycelium is luminous.

Pleurotus japonicus. Since the finding of Fungus (*Pleurotus*) igneus by Rumphius on the island of Amboina in the seventeenth century, many species of *Pleurotus* have been described from tropical countries in the middle nineteenth century under the name of *Agaricus* by Hooker, Gardner, Drummond, Berkeley, Gaudichaud, and others. The reports, like that of Collingwood (1869), undoubtedly refer to species of *Pleurotus* and merely speak of a soft pale glow from fungi abundant in the jungle, in this case in Borneo. *Pleurotus lampas* of Australia is shown in Fig. 34.

One of the best-known species is *Pleurotus* (*Lampteromyces*) *japonicus*, studied by Kawamura (1915). It is a poisonous species from Japan, called locally "tsukiyo-take" or moon-night mushroom, which grows on the dead trunks of beech trees in September and October and closely resembles the non-luminous and non-poisonous *P. ostreatus*. The luminescence comes only from the gills of the fruiting body, not the mycelia, beginning at the stage when the pileus is fully expanded. The light lasts until the plant is quite old, fading away

gradually. Cells of the hymenium-subhymenium and trama are luminous but not the spores.

McAlpine (1901) has reported that only the gills of *P. candescens* of Australia are luminous and the mycelium only for the first two days, and Bose (1935) has described a *Pleurotus* sp. from South Burma whose fruiting body was entirely luminous (stalk and both surfaces of pileus). He was able to grow a mycelium which was also luminous. Haneda (1939, 42) found *P. luminaustris* to be widely distributed in tropical Asia. The fruiting body and mycelium are luminous but the spores are not.

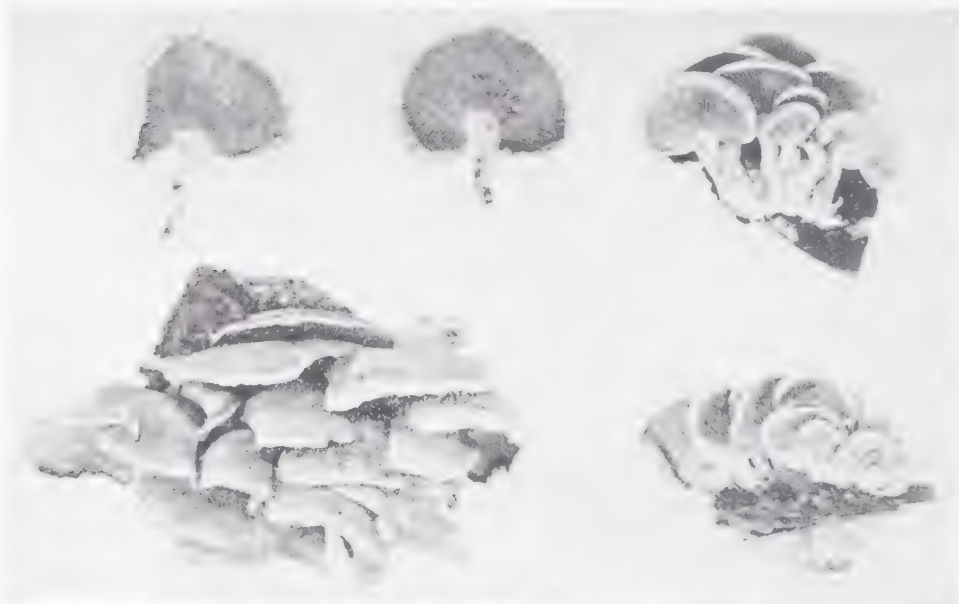


FIG. 29. *Panus stipticus*. Photograph by J. B. McCurry, supplied by Ruth Macrae.

Panus stipticus. This wood-destroying fungus, shown in Fig. 29, appears to have been first recorded as luminous by Ellis (1886), who found the gills but not the stipe or top of the pileus or the wood near the fungus to emit light. Not all specimens that he collected were luminous and the light "seemed to depend on some peculiar condition of the air, having been noticed only in specimens gathered in damp weather or just before a storm." Atkinson (1889) and Murrill (1915) also saw the luminescence and Murrill noticed that the portion of the mature fruiting body which bears the hymenium was luminous.

The most extended observations are due to Buller (1924). He was able to revive dried fruit bodies from Montreal, Canada, by moistening with water six weeks after collection and observed the bright greenish luminescence, strongest on the gill surface but also on the

upper surface of the pileus, but not the stipe. The flesh of the pileus may glow faintly. In young fruit bodies the stipe is also luminous, but as they grow older light emission ceases from the stipe, the upper surface of the pileus and finally from the gills in the order named. The spores are not luminous, even when moistened and about to germinate. The early developed hyphae are also not luminous, but after a certain amount of growth (culture 3 cm diameter) light appears.

The most interesting aspect of *P. stipticus* is the existence of two varieties, a North American luminous form and a European non-luminous variety, both morphologically alike yet physiologically distinct. The mycelia are able to fuse with each other, and Buller suggested that these varieties presented an opportunity to find out if the secondary (diploid) mycelia resulting from crossing two primary (haploid) mycelia of opposite sex might be (1) always luminous, (2) always non-luminous, or (3) sometimes luminous and sometimes non-luminous. These genetical experiments have since been carried out by Macrae.

Omphalia flavida. Another luminous fungus, described at great length by Buller (1934) is *Omphalia flavida*, first noticed to be luminous by Buller and Vanterpool (1926). Another luminous species from Borneo, *O. martensii*, was known to Hennings (1893). These are leaf-spot fungi, attacking a great variety of plants, causing a serious disease of coffee leaves in Trinidad. The mycelium is luminous but not the pileus or the gemmae (stilbum bodies) by which the fungus reproduces itself. The leaf spots of *Omphalia* in coffee leaves are luminous but another coffee fungus, *Sclerotium coffeicola* is non-luminous. *Omphalia* also attacks growing leaves of *Borreria ocymoides* in the tropical rain forests of Puerto Rico, causing brightly luminous white patches, shown in Fig. 30, as the mycelium penetrates the leaf tissue.

Luminous Leaves, Peat, and Mold, the Genus Mycena. The luminescence of mold and peat appears to have been first noticed in 1686 by Plot, who gave a vivid account of the light from the hoof marks of horses crossing a peaty bog in wet weather. Damp dead leaves also become luminous, as described by Naudin (1846). The phenomenon is very common among decaying debris in dark damp forests and has been specially studied by Tulasne (1848), Molisch (1904, 12), Buller (1924), Bothe (1931), and Wassink (1948). Both Molisch and Buller had no difficulty in obtaining luminous leaves from forests in Java, Germany, England, and Canada. Molisch (1915) also found them in Japan.

Bose (1826) noticed that leaves, stalks, grass roots, and living roots of *Averrhoa carambola* from the forest bed in Barisal, Bengal, were luminous. He found fungal hyphae among them and concluded that a

fungus must be the cause of the light, since drying and other agents which affect fungi also affected the luminescence of the leaves. Dr. T. Dobzhansky of Columbia University has informed the author that luminous leaves on the floor of tropical rain forests near Santos, Brazil, present a really superb spectacle at night. Luminous roots of tormentilla were observed by Kortum in 1795, and garden lettuce roots (Anon., 1832) have been described, no doubt a luminous fungus infection.

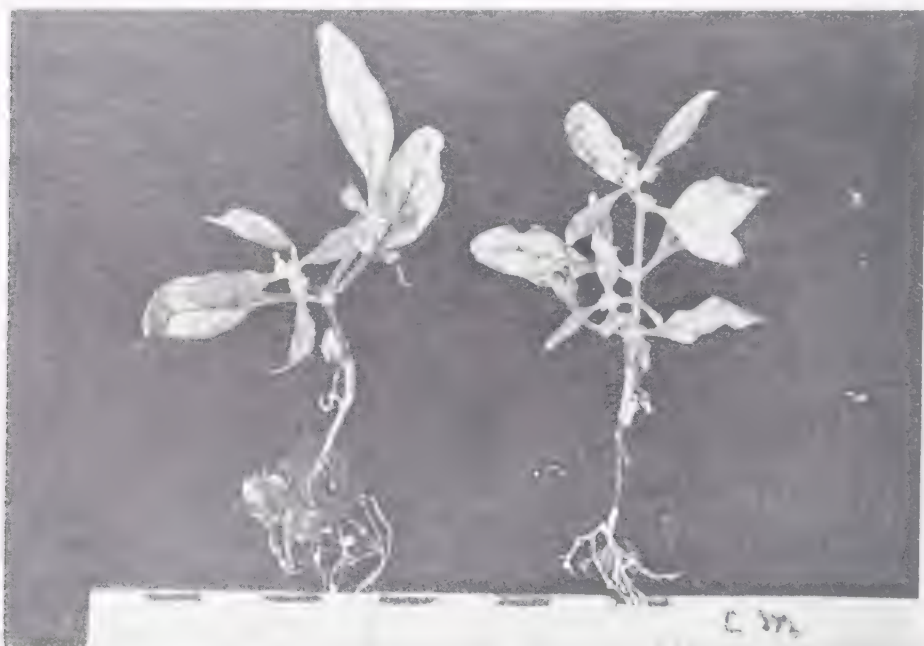


FIG. 30. *Omphalia flava* growing on *Borreria ocymoides* in Puerto Rico. Photo by J. van Overbeek.

Molisch was unable to determine the fungus responsible for the brownish or colorless hyphae found on beech and oak leaves, as they would not grow on artificial media, and Buller, also, made no culture studies. However, Bothe (1931), from pure culture growth decided that the light of leaves might be due to various species of *Mycena*, *M. galopus*, *M. sanguinolenta*, *M. epipterygia*, *M. dilatata*, *M. stylobates*, *M. zephira*, and *M. parabolica*.

The genus *Mycena* is a very large one. *M. tintinnabulum* and *M. polygramma* are luminous wood-destroying fungi, whereas *M. galericulata*, *M. vulgaris*, *M. metata*, *M. janthina*, *M. crocata*, *M. haematopus*, and *M. alkalina*, all grown in pure culture, are not luminous.

* *Mycena galericulata* var. *calopus* = *M. inclinata* is luminous, according to the observations of Wassink (1948).

Of the allied genus *Collybia*, *C. longipes*, *C. fusipes*, *C. velutipes*, *C. cirrbata*, and *C. tuberosa*, grown by Bothe, are also non-luminous. *Mycena illuminans* of Java is brightly luminous, according to Hemmings (1903).

Wassink (1948) has added another *Mycena* to the group attacking leaves. He found a luminescent spot on an oak leaf which had developed tiny sporophores, allied to *Mycena capillaris*. *M. capillaris* is not luminous, and the oak leaf *Mycena* of Wassink could not be grown on cherry agar, but nonetheless he felt sure the luminous spot was due to a very small species of *Mycena*. Apparently the mycelium of these fungi does not readily form fruiting bodies on leaves.



FIG. 31. *Mycena rorida* var. *lamprospora*, by daylight. Photo by Y. Haneda.

Haneda (1939, 42) has described a number of interesting species of *Mycena* from Micronesia and other islands of southeast Asia: *M. bambusa*, *M. phosphora*, *M. noctilucens*, *M. Yapensis*, *M. microillumina*, *M. cyanophos*, *M. citrinella* var. *illumina*, and *M. rorida* var. *lamprospora*, shown in Fig. 31. The latter is of special interest because the fruiting body itself is not luminous and the mycelium is very faintly luminous but the spores are brilliant and responsible for a greenish light from moist ground on which they fall. *Marasmius phosphorus*, found on dead leaves in Palao, is also luminous.

Basidiomycetes, Phallales

Three members of the order of Phallales (originally Phallaceae, a family of the Gasteromycetes) have been reported as luminous.

According to Molisch (1912, p. 50) one of these from Brazil, *Dictyophora phalloides*, was described by Möller as "the lady with the white veil," and botanical writers had interpreted the description as indicating luminosity. The inference was incorrect, as Molisch had the assurance of Möller himself that *Dictyophora phalloides* is not luminous.

The record of luminescence of *Ileodictyon* (*Clathrus*) *cibarium*, another of the Phallales, is somewhat obscure. Molisch (1912, p. 31) says that he found a casual reference to its luminescence in R. Ledentfeld's (1887) "Die Leuchtorgane der Fische." In this work the name is given as "*Ileodiction cerebrum* ein Pilz," but no reference is given. Ludwig (1890) used the same spelling. Klein (1928) places *Ileodictyon cibarium* among the luminous fungi, indicating that it is found in Australia, New Zealand, and South America, but also gives no reference. It is not included in the McAlpine (1901) list of luminous Australian fungi, based largely on the compilation of Zopf (1890). Wassink and the author have been unable to find recent observations, and the luminosity of this fungus is highly doubtful.

The only other member of the Phallales which may be luminous is *Kalchbrennera corallocephala* from Africa. It is mentioned by Ludwig (1890) and is included in the Molisch (1912) and Klein (1928) list, but without a reference. Wassink and the author have been unable to trace the original report, and *Kalchbrennera* luminescence must also be considered very doubtful.

GENETIC STUDIES

The first studies on interbreeding of luminous fungi were made by Bothe (1935), who grew the luminous leaf fungus, *Mycena galopus*, from single spores of two fruiting bodies and the luminous wood fungus, *Mycena polygramma*, also from single spores. He observed that in both species some of the primary mycelia from single spores might be luminous and others non-luminous. A few were of low luminosity. The same was true of the secondary diploid growths, resulting from fusion of haploid mycelia. It was thus demonstrated that these species of *Mycena* occurred in luminous and non-luminous forms, just as in the case of *Panus stipticus* of European (non-luminous) and North American (luminous) origin. Bothe concluded that luminescence is affected by three series of factors, some of which serve as inhibitors and at least two of which are composed of multiple allelomorphs. The results were difficult to explain since basidiocarps were not obtained from the crosses, and the study was confined to mycelium of the first generation.

Complete genetic studies on *Panus stipticus* *luminescens* and non-luminescens have been made by Macrae (1937, 42) in relation to pairing reactions and the inheritance of the factor for luminosity. Pairings of monosporous mycelia from both types show that they are heterothallic and tetrapolar and completely interfertile. The diploid

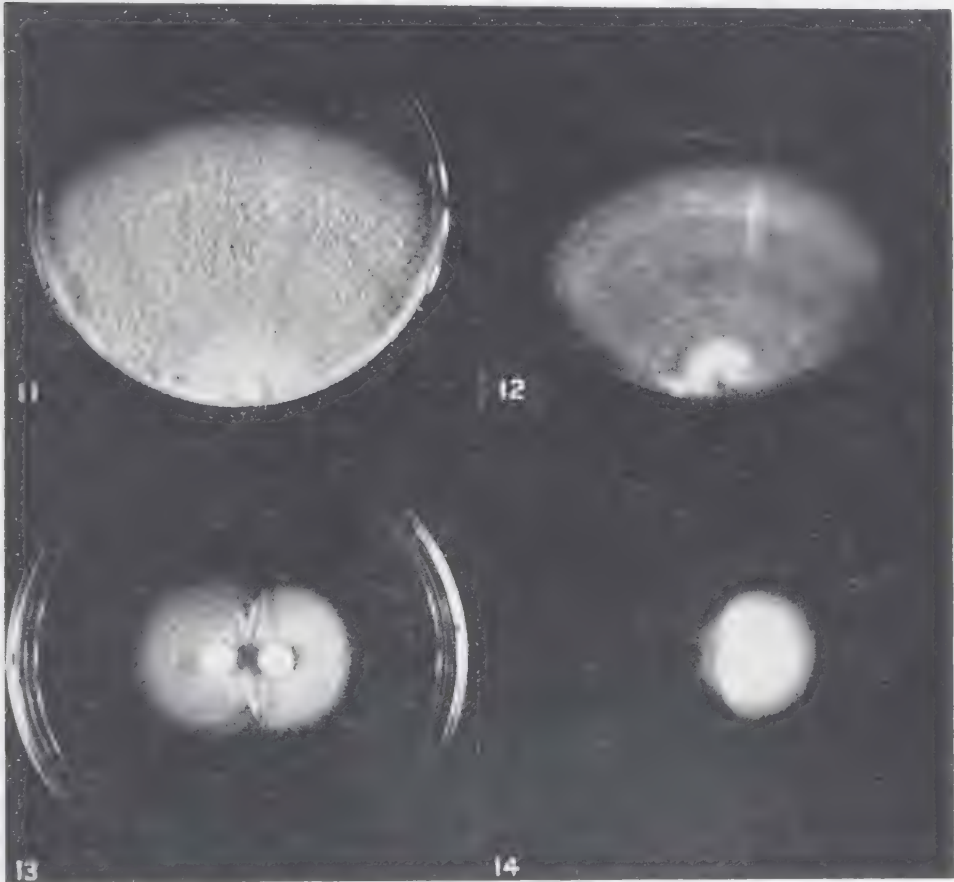


FIG. 32. *Panus stipticus* cultures (4 weeks old) on malt agar. (11) Diploid mycelium from pairing (4 weeks old) of luminous American and non luminous European haplont strains, photographed by reflected light. (12) Same photographed by its own light. (13) Pairing (2 weeks old) of non luminous haplont (to left) and luminous haplont (to right) by reflected light. (14) Same, photographed by its own light, showing the luminous diploid mycelium in the area between the two inocula. Photo from Ruth Macrae.

mycelium and fruit bodies produced in the F_1 generation are always luminous. Studies on the haploid mycelia from an F_1 fruit body indicate that luminosity is inherited as a single pair of characters, "dominant over non-luminosity and that the luminosity factors form all possible combinations with infertility factors." Some of the cultures are shown in Fig. 32.

HISTOLOGY

The chief attempt to study the cytology of luminous fungal hyphae is that of Dahlgren (1916), who published a section of a cap of *Clitocybe illudans* showing very small nuclei and cell vacuoles, presumed to be the seat of luciferin secretion. Further studies with modern techniques should be undertaken. Dahlgren's drawing is reproduced as Fig. 33.



FIG. 33. Section of the cap of *Clitocybe illudans*, showing very small nuclei and vacuoles. After Dahlgren.

NUTRITIONAL REQUIREMENTS

Luminous fungi grow readily on artificial culture media, either spores, fragments of fruiting body, rhizomorphs, or mycelium serving as inoculation material. Such simple culture media as cherry agar or bread agar contain all the necessary ingredients for growth and luminescence, but on bread agar, according to Wassink's (1948) experience, the light is somewhat brighter and Molisch (1904, 12) also recommended bread agar. Bread agar is made up of 10% dried bread crumbs and 1.8% agar in tap water. Cherry agar is prepared by cooking 1 kg of cherries in 1 liter of water, mashing the cherries and passing the material through a sieve with 2-sq mm holes. One part of this extract added to 2 parts of 2% agar forms the culture medium.

Bothe (1928) used 10% bread and 1.8% agar or 10% gelatin, but controlled the nutrient and salt content of the medium more carefully. He also used a pure nutrient medium of 0.25% MgSO_4 , 0.25% KH_2PO_4 , a trace of FeCl_3 , 5% sugar, and 1% peptone in distilled water with 1.8% agar or 10% gelatin. Molisch used a nutrient medium of 10% gelatin, 3% saccharose, 0.6% NH_4Cl , 0.05% MgSO_4 , 0.05% KH_2PO_4 , and a trace of iron. Fungi will also grow well on sterilized blocks of damp beech, birch, or oak wood.

Using such techniques, a systematic study of pure cultures has been made by Molisch, Buller, Bothe and Wassink. Molisch has grown luminous *Armillaria mellea* and *Mycelium X* and non-luminous *Xylaria hypoxylon* and *X. cookei*, *Trametes pini* and *T. radiciperda*, *Polyporus sulfureus*, *Collybia cirrhata*, and *Panus stipticus* while Buller grew *Panus stipticus luminescens* and *P. stipticus non-luminescens* (1924) and *Omphalia flavida* (1934).

Bothe has grown *Agaricus (Armillaria) mellea* and *Mycelium X* (1928), *Clitocybe olearia* (1930), *Mycena tintinnabulum* (1930), and 19 other species of *Mycena* and *Collybia* (1931), some luminous and some not, while Wassink (1948) has cultivated luminous *Armillaria mellea*, *Clitocybe olearia*, *C. illudens*, *Omphalia flavida*, *Mycena galopus*, *M. epipterygia*, *M. polygramma*, *M. tintinnabulum*, *M. inclinata*, *M. galericulata* var. *calopus*, *M. parabolica*, *M. sanguinolenta* and *Polyporus rhipidium*. The brightest luminescence was observed in *A. mellea*, *Mycena polygramma* and *Omphalia flavida* grown on bread agar. The non-luminous species grown in pure culture by Wassink were *Panus stipticus* (a strain of Siberian origin), *Mycena epipterygoides*, *M. capillaris*, *Collybia tuberosa*, *C. cirrhata*, *Clitocybe tabescens*, and *Polyporus sulphureus*. It will thus be seen that a very large variety of fungi is available for luminescent work, most of them maintained by the various national culture collections.

The most extensive paper on metabolic requirements is that of Bothe, who started working on fungi in Molisch's laboratory in Vienna with the idea of comparing the nutrients and general conditions for light production with those necessary for luminous bacteria. The first studies were with *Armillaria mellea* and *Mycelium X*, and the chief conclusions were as follows: Glycerine is more effective in promoting luminescence than fructose for *Mycelium X*, but the converse relation holds for *A. mellea*; both are more effective than saccharose. Promotion of growth is in the order fructose > glycerine > saccharose for the two fungi. In a number of respects growth and luminescence are independent processes.

No particular osmotic concentration of salts is necessary for light

production, but 0.5 to 1% of the alkali chlorides promote luminescence, K more effectively than Na. Zn stimulates growth and luminescence in very small concentration, Cu only temporarily, Fe not at all, and Mn in such high concentration that "stimulation" cannot be spoken of.

Foreign fungal or bacterial growths do not increase the light intensity of cultures, but deal fungal material of *Psalliota campestris* increases the light intensity of Mycelium X. Wounding the mycelial threads of this growth also increases the light. The effect begins about ten minutes after wounding, reaching a maximum in five to eight hours, and then gradually decreases over a three-day period. There were no essential differences in the growth or luminescence behavior of Mycelium X and *A. mellea* except in the strong rhizomorph formation of the latter fungus.

Hamada (1940) has made an extensive study of growth conditions for *A. mellea* with special reference to oxalate formation but little attention to luminescence. He observed that maximum luminescence and guttation occurred between an N:C ratio of 1:2 and 2:1.

Growth and luminescence studies with nutrient media containing known simple essential constituents and the production of mutants lacking the ability to synthesize certain compounds have not yet been attempted with fungi. Because of the slow growth of fungus mycelium and the low light intensity they are not as favorable for study as the luminous bacteria.

RELATION TO OXYGEN

Since Boyle's famous experiment in 1667 with his air pump and a piece of shining wood, in which he showed that the light disappeared in a vacuum, practically every experimenter has studied lack of oxygen, either by evacuation or replacement by neutral gases. Toward the end of the eighteenth century groups of investigators, stimulated by the chemical discoveries on gases and combustion, investigated the effect of gases on luminous wood. The papers of Achard in 1785, Spallanzani in 1796, Carradori in 1796, Tychem in 1797, vom Humboldt in 1799, Gaertner in 1799, Hulme (1800, 01), and Boeckmann (1800) all deal with the effects of oxygen. Their work was also referred to in the early prize essays of Bernoulli (1803), Link (1808), Heinrich (1808, 20), and Dessaigues (1809).

It is interesting to note that the above workers were about equally divided in their conclusions regarding the necessity of oxygen for light production, some obtaining light in hydrogen or nitrogen because of the presence of small amounts of oxygen as impurity. All the more recent workers on various forms—Archangeli, 1889, Kawamura, 1915,

Buller, 1924; Nobecourt, 1926; Harvey, 1926; Bothe, 1928) find an extinction of light in N_2 , H_2 , or CO_2 and return of light in the air, provided the fungus has not been deprived of oxygen too long. The work of Hastings (unpublished) in the author's laboratory has indicated that the curve relating luminescence intensity and oxygen pressure is similar to that of luminous bacteria, but the oxygen pressures to reduce the light the same amount are 100 times greater for the fungus, *Armillaria mellea*, than for a marine luminous bacterium of unknown strain.

Those who have tested the luminescence of fruiting bodies in pure oxygen (Kawamura, 1915; Buller, 1924) report no change in light intensity, but a mycelium growth or luminous wood may be in partial lack of oxygen in the air, due to slow diffusion to crowded hyphal regions. Under such conditions pure oxygen will increase the light intensity, as Bothe's (1928) experiments have indicated.

When Boyle readmitted air to his "shining wood" in a vacuum, he thought the light revived a little brighter than before. In fact unpublished observations of F. H. Johnson and W. D. Lynn (1940) indicate that Boyle's observation was correct. The mycelium of *Panus stipticus* was used and hydrogen gas replaced the air. Photometer measurements indicated increased luminescence similar to that of luminous bacteria to which oxygen is supplied after a period of anoxia, but the time scale was about 10 times longer. Instead of a flash of two seconds, as in bacteria, the excess luminescence of the fungus lasted twenty seconds. Temperature had an effect similar to that on the flash of bacteria, low temperatures reducing the intensity and prolonging the duration of the excess light, while higher temperatures (up to a limit) had the opposite effect.

EFFECT OF TEMPERATURE

No quantitative studies on light intensity and temperature have been made, but early observers, for example Hulme (1800, 01), noted that luminous wood still glowed near the freezing point of water and that the light went out at high temperatures.

A few determinations of minimum, optimum, and maximum temperatures have been made. Kawamura (1915) found lower and upper limits of 3–5° and 40° with an optimum region 10–15° C for *Pleurotus japonicus*, and Buller (1924) gave –2 to –4° C and 35–37° C, with an optimum range 10–25° C for *Panus stipticus* luminescens. The curves would undoubtedly look like those obtained with luminous bacteria. There was some recovery of light in both fungi on cooling after the

light had disappeared at the maximum temperature, showing the existence of a reversible thermal process, as in bacteria.

Bothe (1928) studied temperature effects on the growth and luminescence of Mycelium X and *A. mellea* over long time periods. Temperatures of 31–34°C were definitely harmful and 15°C seemed optimal, but the light intensity increased in Mycelium X up to about 24°C and in *A. mellea* up to 18–20°C.

EFFECT OF LIGHT

Like bacteria, the light of fungi is continuous night and day, independent of stimulation, and changing only with age or cultural conditions. The report of Murrill (1915) that *Clitocybe illudens* was less luminous during the day is undoubtedly due to light adaptation of his eyes. Kawamura (1915) convinced himself that *Pleurotus japonicus* exposed to daylight was as bright as specimens kept in the dark, and neither Buller nor the author (1926) nor Coblentz and Hughes (1926) observed any effect of sunlight on the luminescence of wood.

However, Bose (1930) has reported an effect of light on a fungus growing on *Sterculia* wood from Buxa Duar in Jalpaiguri, India. When kept for a number of days, the luminescence gradually disappeared but could be revived by exposure to sunlight, even for as short a time as five to ten minutes, and longer exposures gave a brilliant revival of light. Bose attempted to find the spectral rays responsible for the effect by interposing Wratten filters, but behind all filters there was no effect, probably due to the high absorption of the filters. It is possible that heating of the wood or some change in moisture conditions around the wood might be responsible for the sunlight effect. The shorter visible and ultraviolet rays, if sufficiently intense, would undoubtedly extinguish fungal luminescence.

EFFECT OF DRUGS

Alkaloids. Practically no studies have been made of the effect of oxidative inhibitors, various fungicidal agents, or special drugs on the light of fungi, in contrast to the voluminous literature dealing with drugs and luminous bacteria. Krukenberg (1887) studied strychnine, caffeine, nicotine, and quinine effects on *Agaricus olearius*, but only one concentration was used, and the results are hardly worth recording. He found no effect of distilled water, strychnine and caffeine, weak stimulation by nicotine, which soon became inhibiting, and a direct inhibiting action of quinine. Alkalies strongly stimulated the light intensity and then inhibited.

Anesthetics. Early studies on narcosis of light production among fungi also suffered from lack of control of concentration. Krukenberg

(1887) reported that chloroform stimulated at first and then inhibited luminescence of *Agaricus olearius*, and Kawamura (1915) also found that with old specimens of *Pleurotus japonicus*, in which the light was already weak, chloroform and ether vapor increased the light at first and then gradually extinguished it. Bright specimens of *Pleurotus* gradually become more and more dim in chloroform and ether vapor. When the light disappeared it could not be revived on removal to air.

On the other hand Buller (1924) observed a true reversible narcosis of luminescence in fruiting bodies of *Panus stipticus luminescens* in chloroform and ether vapor, and Nobecourt (1926) found the same behavior with *Armillaria mellea* mycelium. Lutz (1931) compared the effect on *Armillaria* of ether, benzaldehyde and other antioxidants which quench the luminescence of Delepine's organic sulfur compounds. The vapors reversibly extinguished the fungus light, leading Lutz to the conclusion that fungus luminescence was an autooxidation.

BIOCHEMISTRY

Several investigators (Ewart, 1907, on *Pleurotus candescens*; Kawamura, 1915, on *P. japonicus*; Buller, 1924, and Harvey, 1926, on *Panus stipticus luminescens*) have endeavored to obtain luminous juice from fungi but without success. Crushing the cells always destroys the light.

Buller found that if dried *Panus stipticus* was powdered in a mortar and then water added no light appeared, although the whole dry fungus will emit light if it is revived by moistening. As in the case of bacteria, the intact fungal cell appears to be necessary for light production. The author determined that *P. stipticus*, like luminous bacteria showed no special fluorescence in ultraviolet light.

The first attempt to demonstrate the luciferin-luciferase reaction was made by Ewart (1907), who applied the test to "unusually fine specimens" of *Pleurotus candescens* but with negative results. The author, (1926) using *Panus stipticus*, and Bose (1935), with a *Pleurotus* from South Burma, have also failed with the luciferin-luciferase reaction. Even if the *Panus stipticus* luciferin is prepared in absence of oxygen and then added to an extract of the fungus, the author observed no luminescence.

Little work has been done on other enzymes in relation to luminescence. Nobecourt (1926) found an oxidase which caused gum guaiac to turn blue in the sap from *Armillaria* cultures. He thought this oxidase might be concerned in luminescence since light appeared in young cultures about the same time as browning began, but there is no evidence that oxidases are involved in bioluminescence.



FIG. 34. *Pleurotus lamps* of Australia, photographed by its own light (above) and by daylight (below). Photo by Mr. Allan Foott, Turramurra, Australia.

PHYSICAL PROPERTIES

The only studies of light intensity have been of a very inaccurate nature, such as reports of the ability of the observer to read fine print or to see fungal light at a distance of so many paces. Actually the light is not very bright, not nearly as bright as a well-aerated culture

of luminous bacteria and the term brilliant applied to fungi or luminous wood is purely comparative.

Many have used the light to take photographs, usually shadow-graphs of leaves or letters such as those of Kawamura (1915) and Buller (1924, 34). Actual photographs of the cultures have been made by Bose (1935), who reproduced his photograph of a mycelium in a flask grown from rhizomorphs of an Indian fungus similar to *Armillaria mellea*, and by Haneda (1939, 49), who has photographed various species of *Polyporus*, *Mycena*, and *Omphalia*. In Fig. 34 there are reproduced living specimens of *Pleurotus lampas* from Australia, photographed by their own light.

The spectrum of luminous wood was first studied by Achard in 1785, who, because of a poor instrument, thought it monochromatic. Ludwig (1884) demonstrated that fungal light, from what he identified as *Trametes pini*, *Agaricus melleus*, and *Xylaria hypoxylon* (both rhizomorphs and mycelial growths) and *Collybia tuberosa* sclerotia, extends from the orange to the blue, and Molisch (1904, 12) also found a similar short band from mycelium X. Macrae (1942) obtained spectral photograms of *Panus stipticus* extending from 490 to 580 $m\mu$.

The spectral energy curves have been obtained by Coblentz and Hughes (1926) and by van der Burg (1943). The first-mentioned authors studied *Agaricus melleus* mycelium, exposed for 24 and 71 hours before the slit of their spectograph. The spectrum extends from 470 $m\mu$ to 680 $m\mu$ with a maximum at 520 $m\mu$. Van der Burg obtained curves for *A. mellea*, *Mycena polygramma*, and *Omphalia flavida*, which were practically identical, with a maximum at 528 $m\mu$, shown in Fig. 24. Finally, Haneda (1939) has photographed the spectrum of *Polyporus hanedai*, *Mycena bambusa*, and *Pleurotus lunailustris* and recorded the blackening of the film with a micro-photometer. These spectra were also alike, extending from 449 to 658 $m\mu$, with a maximum at 520 $m\mu$.

CHAPTER III

Protozoa and Porifera

PROTOZOA

CLASSIFICATION

Among the great group of one celled animals, only the salt water dinoflagellates (Dinophyceae or Peridineae) and the exclusively salt water radiolarians contain luminous forms. The position of these two orders among the six classes of Protozoa in the classification of L. Rhumbler and V. Jollos is as follows:

Rhizopoda or Sarcodina

Amoebozoa (10 families, fresh and salt water, parasites)

Reticulosa or Foraminifera (45 families, mostly marine, a few fresh water)

Heliozoa (4 suborders, about 50 species, mostly fresh water, a few marine)

Radiolaria (34 families, marine exclusively)

Xenophyophora (2 families, 22 species, deep sea)

Mycetozoa (3 suborders, Acrasiae, Phytomyxinae, Myxogasteres, terrestrial)

Mastigophora or Flagellata

Chrysomonadina (7 families, salt and fresh water)

Cryptomonadina (2 families including Chilomonas, fresh water)

Dinoflagellata (6 families,¹ salt and fresh water)

Chloromonadina (Gonyostomum, Vacuolaria, Trentonia, Thaumatomastix, on damp ground)

Euglenoidina (3 families, fresh water and parasites)

Phytomonadina or Volvocales (5 families, mostly fresh water, a few marine)

Protomonadina (7 families, parasites, fresh and salt water, including Trypanosoma)

Polymastigina (2 suborders, parasites)

Amoebosporida (5 orders, parasites)

Sporozoa (3 orders, parasites)

Infusoria or Ciliata (4 orders, fresh and salt water)

Suctorina or Akineta (8 families, fresh and salt water)

Since it is a general rule that fresh water or parasitic organisms are not luminous, large sections of the Protozoa may be eliminated as

¹ Some authors recognize as many as 15 families.

expected luminous forms. It is perhaps surprising that among the Foraminifera, predominately marine forms, no luminous species have been described. Although the Heliozoa are mostly fresh water Protozoa, luminescence of the few marine species has never been observed, and among Ciliata also marine species are non-luminous.

The Mycetozoa, usually considered fungi, are prospective luminous organisms, but thus far no luminous slime-molds² have been described. On the other hand, the Radiolaria and particularly the Dinoflagellata, of which the famous *Noctiluca miliaris* is a member, contain some of the most cosmopolitan and interesting luminous animals, whose potentialities for research have not been fully appreciated. They are largely responsible for the diffuse luminescence of the sea.

RADIOLARIA

The emission of light by these forms appears to have been discovered by Tilesius, who wrote an account of the Russian voyage of discovery around the world in 1803-6, under the command of von Krusenstern. A résumé of the results appeared by Gilbert in the *Annalen der Physik* for 1819 with special attention to light-emitting organisms. It is probable that Fig. 23 of plate II in this article is a radiolarian. Baird (1831) has likewise been credited with observing the light of radiolarians but Brandt (1885) considered his drawing questionable.

A little later, Meyen (1834) studied the luminous forms collected on a trip to Canton in a trading vessel. He gave more recognizable figures of the Radiolaria, *Sphairazon fuscum* and what he called *Physaematium atlanticum* and *vermiculare*, all new species. Giglioli (1870) also reported seeing the luminescence of *Thalassicolla*, *Sphaerozoum*, and *Collozoum* in the South Pacific during a world voyage in the *Magenta* in 1865-8. Although frequently studied by Huxley, Müller, Haeckel, Cienkowski, Hertwig, Schewiakoff and others, these zoologists did not observe the luminescence.

The best account is by Karl Brandt (1885) who observed luminous Radiolaria at Naples and published a beautifully illustrated monograph of 276 pages on the subject. Four pages of the work are devoted to light emission. *Myxospaera coerulea*, *Collozoum inerme*, *Collosphaera Huxleyi*, *Sphaerozoum neopolitanum*, *S. punctatum* and *Thalassicolla nucleata* were found to be luminous, and Brandt thought that all radiolarians might be. Figures 35 and 36 show two of these organisms. Over 4,000 species of Radiolaria have been described, mostly from the deep sea.

² A possible exception is *Didymium*. See the discussion under Agaricales.



FIG. 35. A colonial radiolarian, *Collozoum inerme*. After Brandt.

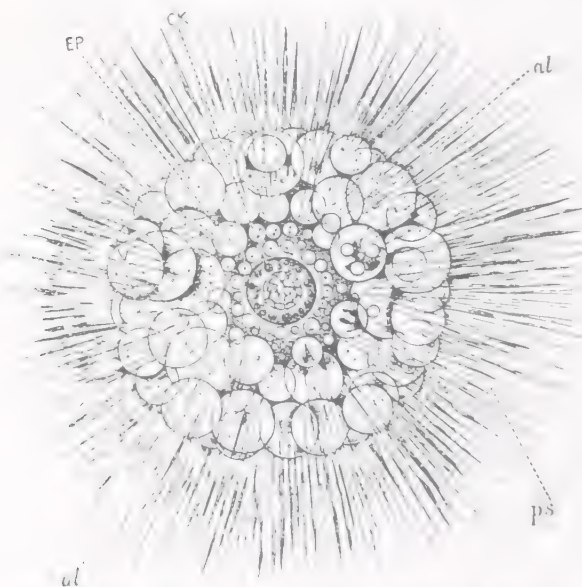


FIG. 36. *Thalassicola pelagia*. CK, central capsule; EP, extracapsular protoplasm; al, alveoli in the mucilaginous calymna; ps, pseudopodia. After Lancaster.

The position of these luminous genera (in italics) in Rhumbler's classification of the Radiolaria is given in the following table

Radiolaria

Acantharia (4 families and 18 genera)

Spumellaria or *Peripylea*

- Sphaeroidae (4 genera)
- Prunoidae (1 genus)
- Discoidae (4 genera)
- Larcoidae (3 genera)
- Sphaerozoidae* (*Collozoum* and *Sphaerouzoum*)
- Collosphaeridae* (*Collosphaera* and *Myxosphaera*)
- Physematidae (2 genera)
- Thalassicollidae* (*Thalassicolla*)
- Thalassothamnidae (2 genera)
- Orthosphaeridae (1 genus)
- Nasselaria or Monopylaria (6 families and 25 genera)
- Tripylea or Phaeodaria (14 families and 39 genera)

Structure

Radiolarians are either solitary or colonial, the colonial forms often growing to very large aggregates. The nutrition is holozoic, although symbiotic "yellow cell" (zooxanthellae) may sometimes be present. The chief structural characteristic is a chitin-like membrane containing holes or pylea between the inner (central capsule) and outer zones of protoplasm. The intracapsular protoplasm contains nuclei, oil particles, and plastids and, according to Brandt (1885), is the seat of the luminescence. He was much influenced by the work of Panceri and Radziszewski and thought that oxidation of droplets of oil in the cells was the source of the light. Under the microscope, Brandt reported being able to see the central part of *Sphaerouzoum punctatum*, the region surrounding the large oil drop luminesce, while the cortical layer (Rindensubstanz) and jelly were dark. The Radiolaria are also characterized by presence of pseudopodia and a skeletal network of silicia or of strontium sulfate, often of the most beautiful and complicated form. The paper by Huth (1913) gives an excellent account of the life history of *Thalassicolla*, the most common luminous species.

Physiology and Biochemistry

Brandt (1885) noticed that colonies of *Myxosphaera coerulea* gave off a weak light when stimulated by gently moving the water in the dish containing them and a stronger light on shaking the water. On continued mechanical disturbance they showed fatigue but would recover and again light on stimulation, if allowed to rest. *Thalassicolla nucleata* lighted more brightly the stronger the mechanical stimulation. When placed in fresh water, all the Radiolaria luminesced for fifteen minutes, increasing in intensity on stimulation, but in twenty minutes the light had disappeared. Brandt also noted that fatigued Radiolaria, which gave no light on mechanical stimulation, would luminesce if placed in fresh water or on chemical stimulation.

In all cases luminescence was weak compared with that of luminous worms and fire flies. The purpose of the light he regarded as a "Schreckmittel," to frighten predacious organisms away.

The author (1926) has studied the luminescence of *Thalassicolla* nucleata and *Collozoum inermis* at Naples and confirmed the various statements of Brandt regarding mechanical stimulation and fatigue. The luminescence is not inhibited by a twenty-minute exposure to bright morning sunlight, nor do they appear to be markedly fluorescent in ultraviolet light, as is true of some luminous forms.

The luciferin-luciferase reaction could not be demonstrated, i.e., a hot water extract of *Thalassicolla* (presumably containing luciferin) will not luminesce when mixed with a cold water extract (luciferase solution) allowed to stand until its luminescence ceased. No light appears when *Thalassicolla* "luciferin" is mixed with *Cypridina* luciferase or when *Thalassicolla* "luciferase" is added to *Cypridina* luciferin. In this respect the *Radiolaria* behave like the majority of luminous forms.

It is in regard to lack of oxygen that *Radiolaria* show unusual behavior. Both *Thalassicolla* and colonies of *Collozoum* will luminesce in sea water containing some platinized asbestos through which pure hydrogen has been passed for forty-five minutes. The platinized asbestos assures absolutely oxygen-free conditions.

As a bioluminescence without oxygen is so unusual, the author (1926) has carried out the test in a more drastic manner in a special vessel with two arms, each with inlet and outlet tubes, so arranged that pure hydrogen could be bubbled through both arms continuously. Since *Radiolaria* luminesce brightly in distilled water, this liquid plus platinized asbestos was placed in one arm and *Collozoum* cells plus platinized asbestos in a small amount of sea water in the other arm. After all oxygen had been flushed out by hydrogen, the liquids were mixed by tilting. The radiolarian luminescence was found to be as bright in absence as in presence of oxygen. Similar behavior has been observed with the medusa, *Pelagia*, and with ctenophores. Attempts to extract luminous material from *Radiolaria* have not been made, and nothing is known of the chemical nature of the photogen.

DINOFLAGELLATES

The Dinoflagellata of the zoologist or the Peridineae of the botanist are predominately marine, with relatively few fresh water species. Whenever the phosphorescence of the sea, viewed from a distance, appears to be homogeneous and devoid of large spots of light, the organisms responsible are undoubtedly dinoflagellates. Examined nearby

in a vessel of sea water the apparently homogeneous light will be seen to come from tiny sparks, each spark representing a minute flagellate. They often develop in huge quantity at favorable seasons of the year. In secluded bays where conditions are just right they may form a permanent culture, and the waters of the bay become famous for "phosphorus" and as a showplace for tourists. One such noted spot used to be the "fire-lake" near Nassau in the Bahamas, a small body of salt water fringed with mangroves and connected with the ocean by a narrow channel. The organism responsible for the light was *Pyrodinium* (*Peridinium*) *bahamense*, only $50\ \mu$ long, studied by Plate (1906). When the channel was widened, conditions changed

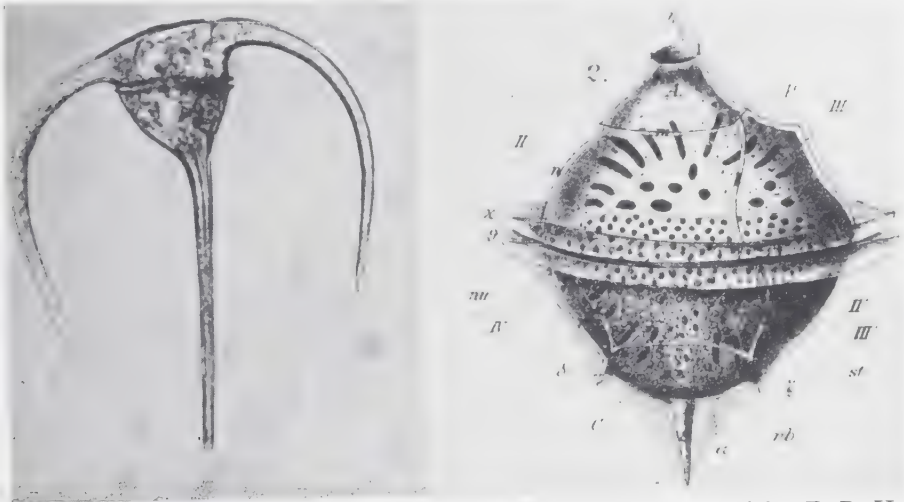


FIG. 37. The dinoflagellates, *Ceratium* sp. (left), photographed by E. B. Harvey and *Pyrodinium bahamense* (right), after Plate.

so that the "lake" no longer served as a good culture medium, and the phosphorescence disappeared. At the present time there is a striking phosphorescent bay (Oyster Bay) near Falmouth on the north coast of Jamaica and another on the south coast of Puerto Rico near Parguera, both surrounded by a growth of mangroves. The author has visited these regions and found the water marvellously beautiful. Every fish that made the slightest movement was outlined with fire, and every wave looked as if it were aflame. Two of the organisms responsible for marine luminescence are shown in Fig. 37.

It is no wonder the "burning of the sea" presented such a mystery to the older observers whose microscopes did not resolve the organisms. The discovery that this homogeneous phosphorescence came always from living things was slow and difficult. Although the luminescence of the largest flagellate, *Noctiluca*, visible to the naked eye, was estab-

lished toward the end of the eighteenth century, the problem of sea light was not definitely settled until about 1830, largely by the labors of Michaelis (1830) and Ehrenberg (1834). They described among others luminescence of the dinoflagellate, *Ceratium*, in the harbor of Kiel, and Ehrenberg (1834) collected all the literature in *Das Leuchten des Meeres* and listed the animals which were responsible for the light. The book of Molisch (1904, 12) contains an excellent account of the history.

There still remains the question as to which dinoflagellates are luminous and the problem of culturing these forms in quantity so that they, like luminous bacteria, will be available for luminescence studies at any time of year. Kofoid and Swezy (1921, p. 52) considered most Peridiniidae and Gymnodiniidae to contain light-bearing species, and Kofoid in a personal note to the author in 1939 suggested that perhaps all dinoflagellates are luminous.

A few observations have been negative. Claparède and Lachmann (1858-1859) and Gourret (1883) were unable to detect dinoflagellate luminescence, but there can be no doubt of light production by many of these forms, at least at certain times of year. In addition to the observers previously mentioned, dinoflagellates have definitely been called luminous in later papers by Ehrenberg (1859, 73), von Stein (1883), Pouchet (1883, 92), Reinke (1898), Molisch (1904), Zacharias (1905), Plate (1906), Czapek (1909), Kofoid (1911), Dahlgren (1915), Cardot and Lefevre (1929), Nakamura (1942), and others.

Colored Sea Water and Luminescence

In many cases dinoflagellates form colored patches on the sea, red, brown, or yellow patches associated with brilliant luminescence at night. In some instances the red color is due to *Noctiluca*, but Torrey (1902) and Kofoid (1911) have found that red water and displays of phosphorescence on the Pacific Coast are more frequently connected with the growth of *Gonyaulax polyhedra*, and Buhegas (1918) observed this same organism accompanied by luminescence responsible for "Hematotalasia" or "blood of the sea" at Pontevedra, Spain. Nishikawa (1901) found *Gonyaulax* to produce brownish yellow water, very luminous at night, in the Bay of Agri, Japan. An unpleasant odor and killing of fishes frequently accompanied the growth or bloom of these organisms. Possibly the red water of the Gulf of California, a region called Vermiglion, Vermijo, or Rojo as early as 1540 by the Spaniards was also due to *Gonyaulax*, for Streets (1878) observed in the red water a small organism that "exploded" under the microscope, but did not mention luminescence. One of the latest

accounts of luminous *Gonyaulax*, together with mass mortality of fish, is by Connell and Barnes (1950) in the Gulf of Mexico near Galveston. The species involved formed chains, as shown in Fig. 38, and appeared to be related to *G. catenella*. Dahlgren (1915) had previously described a colonial "Peridinium," which may have been a *Gonyaulax*, from Delaware Bay responsible for red and luminous patches on the surface.

All instances of red water are not due to *Gonyaulax*. Hirasaka (1922) found that red or chocolate-colored water in Tokyo Bay near Yokohama was due to highly luminous *Gymnodinium sanguineum*.

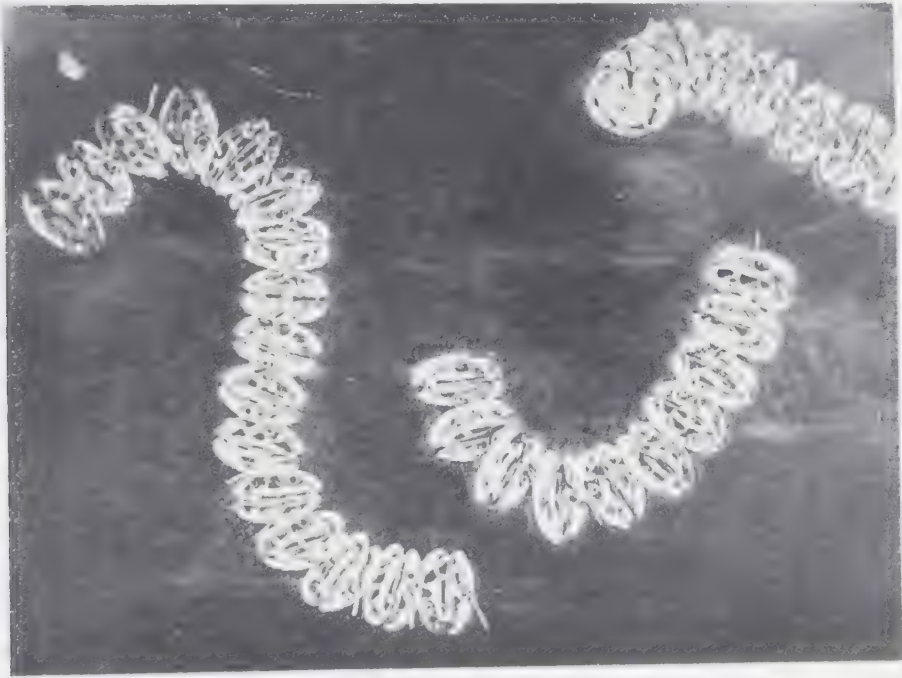


FIG. 38. Colonial dinoflagellates, after Dahlgren.

and Kofoed and Swezy (1921) found yellow water due to luminous *Gymnodinium flavum*.

There are many recorded instances of colored sea water due to flagellates in which no mention of luminescence has been made. The gulf coast of Florida has had visitations of "red tide" for years. The recent one, so destructive to fish along the west coast in 1946-1947, appears to have been due to a number of plankton organisms among which *Gymnodinium brevis* (Davis, 1948) was common (Galtzoff, 1948; Gunther, Williams, Davis, and Smith, 1948). The large yellow-green patches of *Gymnodinium* later changed color to reddish brown. Despite the abundance of *Gymnodinium* no observers of the fish mortality mention luminescence at night and the author made special

inquiries without finding an account of luminescence. Whether this means that no one observed at night or that the organisms are non-luminescent is uncertain.* A compilation of the various reports of red sea water observed since ancient times has been published by Ehrenberg (1830), Dareste (1855), Boué (1869), and Galtsoff (1948, 49). The Boué paper contains a bibliography not only of records of colored sea water but of sea phosphorescence in general.

Possible Luminescence of Fresh Water Flagellates

According to most observers, only marine species of dinoflagellates are luminous, although a few records of fresh water luminescent forms are to be found in the literature. Werneck (1841), a practicing physician at Salzburg, described luminescence in *Ceratium furca*, *Peridinium michaelis* and a so-called *Peridinium lucina* near Salzburg, but Butschli (1885, p. 1022) has doubted the report since these forms are not known in fresh water. Cohn[†] (1856), also, described fresh water luminous flagellates, but Ludwig (1898) after careful investigation has denied their existence. Ludwig found *Peridinium splendormaris*, *P. candelabrum*, *P. eugrammum*, *P. seta*, *Ceratium tripos*, and *C. furca*, all marine forms, to be luminous, while *Peridinium tabulatum*, *P. cinctum*, *P. biceps*, *P. quadridens*, *Ceratium cornutum*, *C. hirundinella*, *Gymnodinium fuscum*, *Amphidinium lacustre*, and *Glenodinium cinctum*, all fresh water forms were not luminous. The list is especially important because it indicates that of two species of a genus one may light and the other not. It would be most interesting to know if the adaptation of a fresh water species to salt water would make it luminous and vice versa. Molisch (1912, p. 22 ff.) also investigated the problem thoroughly and came to the conclusion that "no single fresh water plankton form, either of the plant or animal kingdom was capable of emitting light."

Classification

The chief controversy regarding classification of the dinoflagellates has to do with the position of *Noctiluca* shown in Fig. 40, a genus so unique that a special section is devoted to it. Some authors place *Noctiluca* among the cystoflagellates (see Pratje, 1921), together with *Leptodiscus* and *Craspedotella*, while others, basing their work on

* King (1949) has grown *Gymnodinium simplex* in pure culture and found it non-luminous and has written me that she is doubtful of luminescence in *G. brevis* during red tide outbreaks.

[†] Cohn made no mention of luminescence in fresh water *Peridiniae* in the second edition of his book, *Die Pflanze*, published in 1897.

the studies of Kofoed (1920), place it with the genus *Pavallardia*, among the dinoflagellates proper (Diniferida), in the family Noctilucidae. Kofoed and Swezy (1921) believe that *Craspedotella*⁵ and *Leptodiscus*⁶ are probably luminous, although no records indicate that a definite test has been made, and *Pavallardia* also has not been investigated.

Another difficulty is the luminous genus, *Pyrocystis*⁷ shown in Fig. 39. Some of the brightly luminous species of *Pyrocystis*, dis-



FIG. 39. *Pyrocystis fusiformis* (left) after Gadeau de Kerville; *P. noctiluca* (right) after Chun.

covered on the *Challenger* expedition and described by Murray (1876), resemble *Noctiluca* but are not closely related to it. Thomson and Murray (1885) wrote, "Pyrocystis is strongly phosphorescent, the light proceeding from the nucleus, and it is the chief source of the diffuse phosphorescence of the sea in equatorial regions." Five species of *Pyrocystis* have been listed by Blackman (1902), and Apstein (1909) has added a number of subspecies. However, Kofoed and Swezy (1921) refer to *Pyrocystis* as "a phase in the life history of other Dinoflagellata, e.g., *Gonyaulax*." With these difficulties no classification can be final. The distribution of known luminous genera (in italics) is indicated in the following classification, based largely on Kofoed and Swezy (1921), who recognized a larger number of families than did Rhumbler and Jollos. It is probable that many more genera should be italicized.

⁵ There is no mention of luminescence in Kofoed's (1905) original description.

⁶ Hertwig (1877) made no mention of luminescence in his original description of *Leptodiscus medusoides*, abundant in Messina harbor in winter, but pointed out its similarity to *Noctiluca*.

⁷ Murray (1876) described the genus *Pyrocystis* as a large diatom, giving figures of *P. pseudonociluca* and *P. fusiformis*, which have been copied in most articles on luminescence. Kent (1880) believed *Pyrocystis pseudonociluca* was an encysted form of *Noctiluca miliaris* and *P. fusiformis* an encysted form of *Leptodiscus medusoides*, but J. Schiller has described seven species in L. Rabenhorst's *Kryptogamen-Flora* 10 (abt. 3) pts. I and II,

Dinoflagellata

Adiniferida

Haplodiniidae (Haplodinium) fresh water

Prorocentridae (Exuviella, *Prorocentrum*) marine

Diniferidea

Peridiniidae (*Peridinium*, *Pyrodinium*, *Protoceratium*, *Ceratium*, *Diplopsalis*, *Ceratocarys*, *Peridiniopsis*, *Diplopeltopsis*, *Diplosalopsis*, *Entzia*, *Acanthodinium*, *Goniodoma*, *Gonyaulax*, *Blepharocysta*, *Minuscula*, *Coolia*, *Pyrophacus*, *Oxytoxum*, *Podolampas*) marine and fresh water

Dinophysidae (*Dinophysis*, *Amphisolenia*, *Phalacroma*) marine with some deep sea forms

Phytodiniidae (*Stylodinium*, *Phytodinium*, *Pyrocystis*)

Protodiniferidae (*Protodinifer* or *Protonoctiluca*, *Hemistasia*, *Oxyrrhis*) marine

Gymnodiniidae (*Hemidinium*, *Amplidinium*, *Gymnodinium*, *Gyrodinium*, *Cochlodinium*, *Torodinium*) marine

Polykrikidae (*Polykrikos*) marine

Noctilucidae (*Pavillardia*, *Noctiluca*) marine

Pouchetidae (*Protopsis*, *Nematodinium*, *Pouchetia*, *Proterythropsis*, *Erythropsis*) marine

Blastodiniidae (*Apodinium*, *Blastodinium*, *Chytridinium*, *Elleropsis*, *Oodinium*, *Paradinium*, *Schizodinium*, *Syndinium*, *Trypanodinium*) parasitic forms

Cystodiniidae (*Cystodinium*, *Dinamoeba*, ?*Glenodinium*)

*Amphilothiidae*⁸ (*Amphilothus*)

Gymnasteriidae (*Gymnaster*, *Achradina*, *Monaster*)

?Cystoflagellina

?Leptodiscidae (*?Leptodiscus*, *?Craspedotella*) marine

Day and Night Rhythms of Luminescence

One source of error in declaring dinoflagellates to be non-luminous is light adaptation of the eye. A rest of twenty or more minutes in the dark is necessary in order to observe weak lights. This difficulty has resulted in the announcement of night-day rhythms of luminescence which might be connected with the light or dark adaptation of the eyes of the observer, at least in the case of *Noctiluca*.

Among the smaller dinoflagellates, Zacharias (1905) described a night day luminescent rhythm in *Ceratium* at Kiel but did not mention whether his eyes were always dark adapted at the time of observation. B. Moore (1908) also, at Port Erin Bay, England, studied organisms of the tow, made up of copepods and dinoflagellates but no *Noctilucae*. In one experiment these luminous organisms were kept in a dark room and examined at ten minute intervals from 4:50 A.M. when they were luminescent and the first trace of dawn was showing on the horizon, until 7 A.M. in daylight. As it grew light outside, the luminescence of the organisms gradually disappeared, but Moore did not say whether

⁸ Doubtful flagellates.

he stayed in the dark room during the whole time of observation. Examined at intervals over a twelve-day period these organisms kept in continual darkness would luminesce at night but not in the day.

More recent observations have been made by Kofoed and Swezy (1921, p. 53). "To determine the periodicity of the production of the light, watch glasses containing a single individual, as well as finger bowls containing a quantity of the plankton, were placed in the dark room and tested at various times during the day. These were sometimes taken into the dark room during the day, or as frequently were placed there on being brought in at the 12 and 4 o'clock night hauls. They were thus kept continuously in the dark for twenty four hours. The results were invariably the same in both cases, that is, light ceased to be produced with the early dawn and began again with the coming of late dusk in the evening." Again no mention of dark adaptation of the eyes is made, but the experiments sound convincing.

The author has recently studied the behavior of the dinoflagellates from Oyster Bay in Jamaica, which were collected at night and kept in bottles of sea water in a hotel room. Two bottles were placed in a dark closet and two others on a table near the window. The next day at 3:30 P.M. when the bottles were shaken and observed with thoroughly dark-adapted eyes, there was no luminescence either from those kept in the dark or those exposed to daylight, but later in the evening at 8:30 P.M., all four bottles sparkled profusely when shaken. The next day there was again no luminescence when shaken in daytime, but the sparkling returned at night. After three days the organisms disappeared. The evidence indicates a day-night rhythm of luminescence in these forms, as claimed by previous investigators. In this respect the smaller dinoflagellates differ from ctenophores, whose luminescence is inhibited by strong light but reappears after some time in the dark. The contradictory results obtained in the case of *Noctiluca* will be discussed later.

Use of the Light

The value, if any, of luminescence to unicellular organisms without complex behavior patterns has baffled most zoologists. In the case of photogenic bacteria, where the light is continuous, day and night, it is satisfying to consider the luminescence a fortuitous accompaniment of oxidative respiratory reactions, with no more significance than the production by chromogenic bacteria of a fluorescent pigment. Bacteria can exist without the luminescent process and frequently do mutate to non-luminous strains.

A similar attitude has been adopted regarding the smaller dino-

flagellates and even larger planktonic forms, blown hither and thither by the wind, with practically no ability to determine their own movements or positions. The light has been considered fortuitous, of no value. These organisms do, however, differ from bacteria in that they luminesce only on stimulation and many of them also show a day night rhythm of light production.

A rather novel use of luminescence for such forms has been suggested by Burkenroad (1943), who has regarded the luminescence as perhaps being of no value to the individual but of use to the race. The light emission of a dinoflagellate might attract a copepod which feeds on the dinoflagellate but would also attract a fish which feeds on the copepod, thus destroying the immediate enemy of dinoflagellates. "It is conceivable that the luminescent plankton might at night or in the abyss expose predator to predator along the whole length of the food chain. . . . From the complexity of the food-chain alone, such hypothetical net gain from luminescence would be exceedingly difficult to estimate, but it might nonetheless be real enough." Whether the competition for food is less keen among the population of fresh bodies of water, where luminous dinoflagellates are unknown or whether there is some more directly chemical explanation of the general absence of luminous organisms in fresh water cannot be decided at the present time.

Morphology

Two groups of dinoflagellates may be distinguished, the armored (thecate) and the unarmored. The armored forms are covered with cellulose, often in two pieces with a groove between, each piece made up of numerous plates, sometimes with spines and fin-like ridges. There may be calcareous impregnations of the plates. In the groove lie flagellae but occasionally, as in *Pyrocystis*, they are non-motile. There are frequently red, yellow, green, or brown pigments within the uninucleate cell but many are colorless.

Despite abundant knowledge of the cytology no observation has yet been made on the locus within the cell from which the light appears, except in the case of the large genus *Noctiluca*, where minute granules in the protoplasm are the emitters. We may presume that the smaller dinoflagellates like *Noctiluca* also luminesce from scattered granules within the cell.

Physiology

With the exception of *Noctiluca*, to be considered later, little is known of the physiology of dinoflagellates. Experimentation has been handicapped by lack of pure culture methods of growing the luminous

marine forms. Early attempts in this direction were not too successful, but Gross (1934) has succeeded with *Noctiluca* cultures and later (1937) *Prorocentrum micans* cultures, using the methods of Schreiber (1927). This accomplishment represents a real advance. The growth of luminous dinoflagellates in test tubes like bacteria at all times of the year should initiate a whole new series of discoveries.

The only papers dealing with the physiology of light production in the smaller dinoflagellates are by Reinke (1898) and Zacharias (1905). These men did little more than establish the fact that various species of *Ceratium* would light only when stimulated, mechanically or in some other way. Reinke found that on raising the temperature to 31°C, *Ceratium tripos* began to luminesce and that the light went out at 51°C, but if chemicals were then added, more light would appear, "showing that the animals were not dead." On adding to the sea water such substances as H_2SO_4 , NaOH , ethyl and amyl alcohol, iodine in KI and FeCl_3 , light was stimulated for a short time and then went out. A direct electric current sent through the sea water also stimulated to luminescence.

Zacharias (1905) occasionally noted spontaneous light from *Ceratium tripos* in a dish but thought it due to two animals coming together. He referred to the continuous "rest light," lasting 20 to 30 seconds, which appeared when *Ceratium* was dying. The "rest light" appeared when sulfuric, acetic or chromic acids, K_2CO_3 , KBr , CuSO_4 , FeSO_4 , Na sulfite, uranium nitrate, alcohol, ammonia, formalin, iodine, or AgCl were added to the sea water. The last three substances gave an extraordinarily bright light. This "rest light" is frequently observed when almost any luminous organism is profoundly stimulated and represents an injured condition sometimes called the constant glow or "death glow."

Kofoed and Swezy (1921) have called the luminescence of dinoflagellates silvery white. Certainly it has no bluish tinge as does the luminescence of pelagic ostracods. Attempts were made by Nichols (1924) to determine the brightness of the light, using an optical pyrometer of the type in which an incandescent filament is superimposed upon the glowing surface whose brightness is to be measured. The instrument was calibrated in millilamberts instead of degrees. Since it was practically impossible to superpose the fleeting sparkle of a dinoflagellate and the filament, Nichols observed the filament with one eye and the luminescence with the other. His values were around 0.116 millilambert for dinoflagellates at Woods Hole, Massachusetts, while the brightness of a hydroid colony was 0.033 and that of the ctenophore, *Mnemiopsis leidyi*, from 0.116 to 0.30 millilambert.

Noctiluca miliaris

In many respects *Noctiluca* is one of the most remarkable of living organisms and deserves to become a classic animal in the study of cell physiology. It is not only luminous on stimulation but it possesses a flagellum and a tentacle by which movement can be studied. Its protoplasm bridges a space containing cell sap, which can be analyzed by micro methods. Moreover the animal is large ($1\frac{1}{2}$ to 1 mm diameter), yet contains but a single nucleus; it is nearly spherical and well suited for osmotic studies. In addition *Noctiluca* differs from most marine forms in that its specific gravity is less than the sea water in which it lives, thereby presenting some interesting problems in density regulation.

Despite these peculiar advantages, *Noctiluca* has not been extensively studied by modern experimental methods, partly because of its irregular geographical distribution, partly because it cannot be obtained day after day throughout the year, possibly also from lack of realization of its advantages. *Noctiluca* does not occur in numbers along the Atlantic seaboard, but can be readily obtained along the northern Pacific Coast of the United States. It is abundant in the English Channel, North Sea, in the Mediterranean, Red, and Arabian seas, the Indian Ocean, and eastward to Japanese waters. *Noctiluca* is always a coastal form. The *Challenger*, *Valdivia*, and other expeditions never reported it from the high seas, where *Pyrocystis noctiluca*, the luminous flagellate somewhat resembling *Noctiluca*, is abundant.

There has been some discussion as to whether two species of *Noctiluca* should not be recognized and such names as *N. scintillans* and *N. miliaris* have been given them. The best authorities (see Pratje, 1925) recognize only one form, *Noctiluca miliaris* Suriray. *Noctiluca* may be at times so abundant as to color the water pink or red as described by Krukenberg (1887) near Massaua in the Red Sea and by Hornell (1917) off the Malabar coast of India, where it is known as "flower water" and often precedes great growths of poisonous dinoflagellates called "poison water."

Several interesting observations of green *Noctiluca* have been made—by Weber and Weber-van Bosse (1890) in the East Indies, by Ostroumoff (1924) near Vladivostok, by Peters (1926) near Panang, by Weill (1929) along the coast of Indo China, and by Delsman (1939) in the Java Sea. The green color is due to symbionts which Weill has described as chloroflagellates, dimensions $3 \times 5 \mu$. They swim actively in the vacuolar fluid. Some authors consider the green *Noctiluca* to be another species, since its tentacle is shorter than the Atlantic form, which lacks the green symbionts.

Ostroumoff noticed these green Noctilucae containing motile phytoflagellates in 1909 and again in 1917 in the bay of Vladivostok. He stated that the green form is not luminous whereas the colorless variety, also found in the bay near the island of Askold, does luminesce. As light production is not mentioned by the other writers on the green form, this point should be checked.

Structure. The early history of Noctiluca begins with a letter of Sparshall describing the animal, published in 1753 by Baker in his book, *Employment for the Microscope*. It is probable that Le Roi in 1754, Baster in 1757, and Forskål in 1762 also saw Noctiluca, while Slabber in 1771, the Abbe Dicquemare in 1775, and Macartney (1810) certainly did, for they published easily recognized drawings of the animal, reproduced in a short historical account by Harvey (1940).

The modern period may be said to follow Macartney's 1810 paper and Suriray's description sent to Lamarck in 1810, but not published until 1836. A little later Verhaege (1848) found that phosphorescence of the sea at Ostend was caused by Noctilucae and first recognized the nucleus and tentacular structure. His results, published in 1848, were described earlier by Van Beneden (1846). In the same year Doyere (1846) also called attention to the remarkable organization of this form, and since that time a host of morphological studies^{*} have been made, and the cytology of resting and dividing cells, as well as the method of reproduction, has become well known.

As early as 1850, Quatrefages made a comprehensive study of both morphology and physiology of Noctiluca, a classic paper in the *Annales de science naturelles* which has been twice translated into English. Later workers on morphology and relationships were Busch (1851), Krohn (1852), Gosse (1853), Huxley (1855), Webb (1855), Brightwell (1857), Englemann (1863), Doenitz (1868), Carus (1868), Cienkowski (1871, 73), Allman (1872), Vignal (1878), Robin (1878), Pouchet (1882, 88, 89, 90), Stein (1883), Butschli (1880, 85), Schultze (1886), Griffin (1887), Plate (1888), Ishikawa (1891, 94, 99), Calkins (1898), Doflein (1899, 1900), Kirsh (1909), Fauré-Fremier (1910), E. B. Harvey (1917), van Goor (1918), Kofoid (1920), Pratje (1921), Causey (1926), Hofker (1930), and Gross (1934). Probably the most comprehensive studies on the biology of Noctiluca were those made by Pratje in four papers published with extensive bibliographies in 1921.

The animal is a grooved sphere with a mass of eccentric protoplasm containing a nucleus near the groove and streamers of protoplasm that radiate to a protoplasmic layer around the outer surface. In the protoplasmic strands, granules and food vacuoles can be made out and Fauré-Fremier (1910) and Causey (1926) have described mitochondria.

^{*} Butschli (1883) has given an excellent account.

The light emission is connected with one type of granule. Between the strands is a cell sap, which flows out rapidly when the membrane is punctured. The remains of the animal shrivel, indicating that a certain amount of turgor is present. It is covered with a definite pellicle and osmotic swelling or shrinking occur in hypo- or hypertonic solutions.

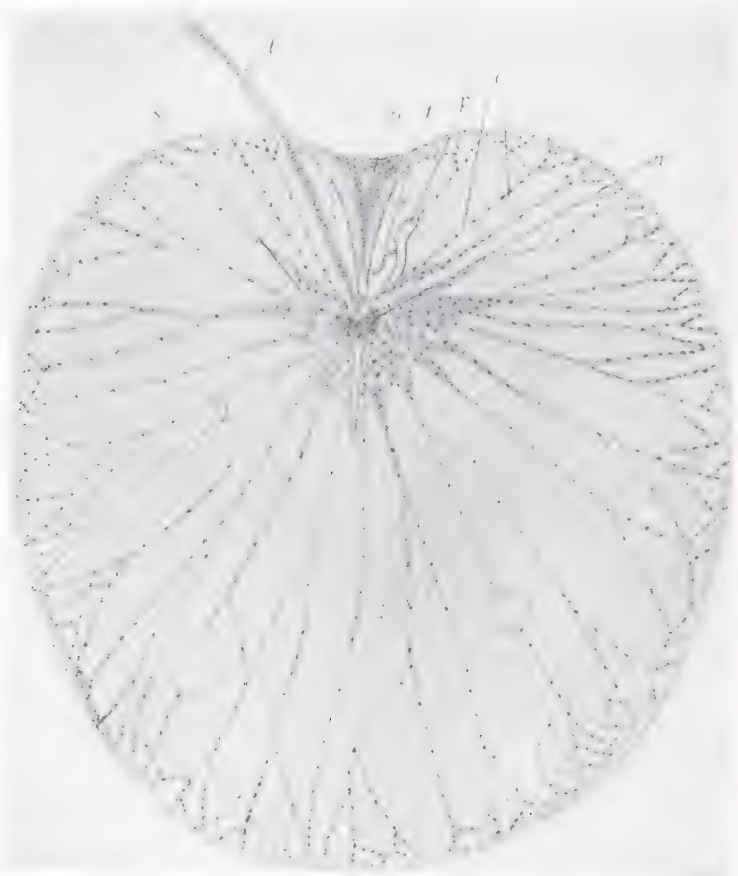


FIG. 40. *Noctiluca miliaris*, showing photogenic granules in cytoplasm. n, nucleus; c, cytoplasmic strands containing photogenic (large) and other (small) granules. p, pharynx; f, flagellum; o, oral groove; t, tentacle; s, spines at base of tentacle; v, vacuoles. Drawn by E. B. Harvey.

The flagellum is minute, and there are complicated structures around the mouth, an oral groove or pouch, a rod and spines at the base of the tentacle. The flagellum undergoes long periods of rest and occasional vibratory motion, not sufficiently strong to move the animal. The tentacle makes slow beating movements, 2 to 4 times or at most 8 to 9 times a minute. Contraction of tentacle appears to be associated with fibrils and cross striation. Figure 40 shows the fine structure of *Noctiluca*.

Noctiluca feeds on various phytoplankton or zooplankton organisms. According to Gross (1934) the tentacle is covered with slime which agglutinates the food into balls which are then shoved into the mouth forming food vacuoles. He succeeded in growing Noctiluca in pure culture on a sea water soil mixture containing NaNO_3 and Na_2HPO_4 and a pure culture of a salt tolerant *Chlamydomonas* on which the Noctilucae feed. Reproduction is by division, about every three days, and also by gametes which conjugate, but Gross was unable to obtain zygotes that would germinate.

Regulation of Density. Many observers, including Macartney (1810), have noticed that Noctilucae slowly rise to the surface of the sea water. Quatrefages makes special mention of this fact and also that they fall to the bottom when the inner sap leaks out. Although the animal possesses a tentacle and a flagellum, these organs are not involved in the movement to the surface. Pratje (1921, 25) and Gross (1934) both agree that the tentacle can do no more than give the animal a rotary motion, and the flagellum is minute. Floating of Noctilucae is the result of its low specific gravity. Massart (1893) found a density of 1.014 and thought the minute oil droplets in the protoplasm gave Noctilucae a low density. However, oil cannot be responsible for their ability to float because, when pricked, so that the internal sap escapes to the outside, or when they die, the protoplasmic remains containing the oil sink. It is the lower density of the sap which is responsible for the ability to float.

One of the earliest studies of this phenomenon was by Goethard and Heinsius, an account in Dutch in a government publication in 1892, which has mostly escaped notice. Krogh (1939) has given a résumé of their work. The Noctilucae just float in diluted sea water of 1.014 density. Sea water itself has a density of 1.024. Allowed to rise through a gradient of diluted sea water the cells increase in size and burst when the density is 1.007 to 1.012. Goethard and Heinsius concluded that osmotic equilibrium is attained but that the sap contains salts of lower specific gravity than those of sea water. They suggested that ammonium salts would fall in this category and demonstrated both ammonia and Cl ion in the sap by microchemical methods. They also found that the Noctilucae would sink in isotonic NH_4Cl but just float in a mixture of 2.9% NaCl and 0.8% NH_4Cl .

Krogh (1937) has agreed with this view and cited Noctilucae as another example of the ability of cells to maintain their internal salt content different from that of the medium. He has pointed out that the high acid content of the sap, whose pH is 3 according to Gross (1934), supplies the condition for ammonium ions to exist within the

cell; otherwise ammonia would diffuse outside. Lyon (1923), Lund and Logan (1924), and Gross (1934) had found that in electrical or mechanical stimulation, acid would pass into the sea water and at the same time the Noctilucae would collapse and sink to the bottom, and Gross has suggested the presence of the acid sap as a necessary condition of the ability to float.

In another early paper on the subject by E. B. Harvey (1917), the density regulation of Noctilucae from Japan was studied in sea water of various dilutions in relation to light emission. In 4 parts sea water and 6 parts fresh water, the Noctilucae sink, but in a short time again rise to the surface. The luminescence response to stimulation is normal. In 3 parts sea water and 7 parts fresh water, there is no adjustment of the Noctilucae. They lose their ability to respond to stimulation by a flash of luminescence, emit a steady glow of light and sink to the bottom. E. B. Harvey explained the low density of Noctilucae as due to a lower salt content than sea water, a view adopted by Ludwig (1928), but this is probably incorrect. The animals undoubtedly come into osmotic equilibrium with the diluted sea water and Iida (1934) showed by Barger's method that the sap pressed out of a dense mass of Noctilucae had the same osmotic pressure as sea water within 5 per cent, whereas the density of the same sap was 80 to 82.5 per cent of that of sea water.

It is interesting to find a stratification of Noctilucae in nature in a brackish water lake of Hokkaido (Japan), Mokotonuma, which empties into the Okhotsk Sea. According to Ueno (1938) this lake has very few Noctilucae at one meter depth, which is nearly fresh water but many Noctilucae at 2 to 3 meters where the salinity is greater. Below this region the Noctilucae fall off again due to lack of O_2 and H_2S generated at the bottom.

Tentacle Movement and Contractility. The tentacle of Noctiluca makes spontaneous slow movements and is also susceptible to various kinds of stimulation, mechanical and electrical. Tentacular response has been studied by E. B. Harvey (1917) who found that at the make of a constant current the tentacle coils up rather tightly, like a watch spring, and at the break it uncoils, the process being repeated for a number of makes and breaks. This behavior is similar to that sometimes observed with the sartorius muscle of a frog. Lyon (1923) showed that the behavior of the tentacle depends on orientation of the Noctiluca. If the current passes from aboral to oral region, the behavior is as described above; if the current passes from oral to aboral there is extreme relaxation at the make (sometimes a slight instantaneous contraction), relaxation while current is passing and a strong instantaneous contraction at the break.

In addition to the tentacular movement the electric current causes contraction and coalescence of the protoplasmic threads radiating from the central mass and breakdown of cell surface films. Lyon (1923) has studied the effect of the electric current on these protoplasmic strands, which results in the formation of blisters or blebs, and found that acid leaked from the cell whenever bleb formation occurred.

Lund and Logan (1925) continued the investigation, showing that breakdown of the protoplasmic films occurs first at the anode, then at the cathode side of the cell. They also demonstrated that the Nernst's equation for relation between intensity i and duration t of a constant electric current, $i\sqrt{t} = k$, a constant, approximately holds for the process of anodal coalescence in *Noctiluca*.

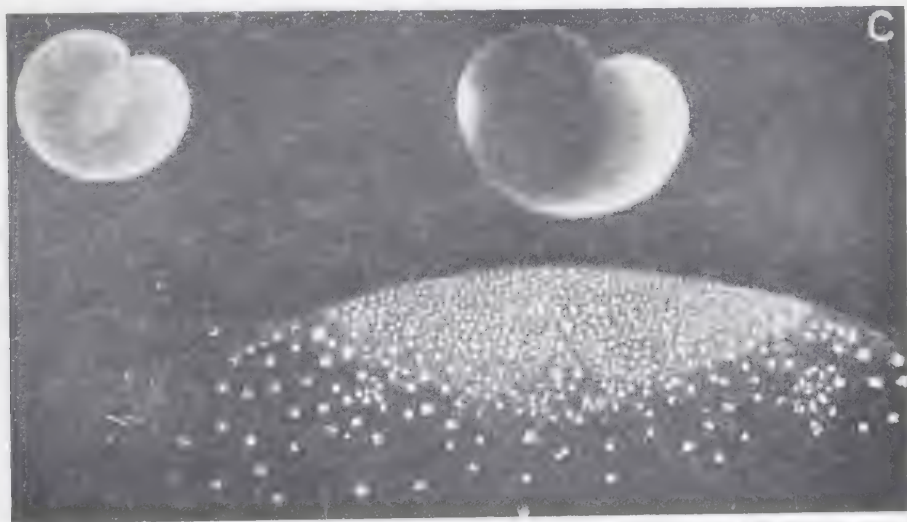


FIG. 41. Quatrefages's famous figure of *Noctiluca*, showing the irregular distribution of luminescence and the points of light coming from granules in the protoplasm.

Luminescence. Two types of light production can be recognized, (1) the steady glow and (2) the flash. Quatrefages (1850) paid particular attention to the steady glow which resulted from prolonged or violent stimulation. He observed under the microscope that only certain regions of the *Noctilucae* would show the steady glow and that it might shift to other regions. Fragments of the animal also exhibited a steady glow, and the light from these fragments or dying animals was similar to that of whole *Noctilucae*. Many bright spots of light could be distinguished, each spot made up of a "cluster of minute instantaneous scintillations, dense at the center and more scattered toward the circumference of the spot." These observations are beautifully portrayed in Fig. 41 of the famous plate accompanying his paper. They have been confirmed by all observers since then.

Stimulation. It was early observed that the animals flash when stimulated by the mechanical agitation of the water and the name, "Medusa scintillans," used by Macartney (1810) to distinguish this small animal from a larger, "Medusa hemispherica," a true luminous jelly-fish, is an indication of the luminous response. The first electrical stimulation to luminescence of minute marine forms, probably dinoflagellates, was made by Pfaff (1823) at Kiel, and the first electrical stimulation of Noctiluca by Pring (1849), who, curiously enough, observed no effect from two "Smee batteries" until the current had been passed for some time, when the Noctiluca gave a constant glow, an effect possibly due to products of electrolysis. Quatrefages (1850) also studied the effects of electricity. Placing the Noctilucae in a porcelain saucer, sheathed in lead, he found that each discharge of a small Leyden jar made the Noctilucae flash, and after three successive discharges they glowed steadily. With a voltaic pile he observed that each time a contact was made, the Noctilucae in sea water in a glass dish would flash and then quickly pass into the state of constant glow. The Zn pole became the center of a luminous circle which widened until the whole surface was luminous from the glowing Noctilucae. This effect was correctly attributed by Quatrefages to decomposition of the salts of sea water, giving rise to acids.

The effects of heat were studied by placing the Noctilucae in a long tube whose bottom was touched to a vessel of hot water. When the convection currents of heated sea water rose through the tube the animals became luminous one by one, and the tube soon presented the appearance of a fiery rod. A similar long tube to which a drop of H_2SO_4 was added "appears to ignite the Noctilucae in its passage as it descends through the sea water."

Quatrefages came to the conclusion that the light was not a secretion, as in pholads and medusae, but was connected with contraction, since all agents which cause contraction of the "sarcode" would also cause luminescence. More specifically he thought the scintillations came from rupture of protoplasmic filaments and the permanent glow from contraction of filaments adhering to the surface envelope of the Noctiluca. Light emission was considered a vital act connected with muscle action, a concept which has persisted in bioluminescence literature for over fifty years.

Later workers of the nineteenth century were mostly concerned with morphology but some physiological experiments were undertaken. Robin and Legros (1866) were astonished at the slight mechanical disturbance necessary to produce light. Observing Noctilucae under a microscope, they noted that a local touch of the surface with a needle

would result in luminescence only in the touched spot; it did not spread to the rest of the animal. In this way all regions of *Noctiluca* were found to be capable of luminescence. Perhaps these experiments should be repeated in order to explore the all or none law in luminescence response and the question of propagated excitation under certain conditions. *Noctiluca* is perfect material for such studies. Later, Robin (1878) took exception to Quatrefages' statement that light production was always connected with contractility, since he had observed that slight vibrations which did not cause tentacle movement or shortening of protoplasmic strands would nevertheless excite luminescence.

Vignal (1878) and Massart (1893) carried out the most comprehensive and extensive studies. Vignal distinguished between the effect of the current in causing retraction of the protoplasmic strands, in stimulating the tentacle, and in causing luminescence, and Massart (1893) considered that the light emission was a response to stimulation, comparable to muscle contraction or gland secretion after stimulation, rather than a result of muscle contraction as Quatrefages had implied. He found that not only would chemical and electrical stimuli cause light emission but also changes in the salt content of the medium, as when fresh water or concentrated salt came in contact with the animals. The fatiguing effect of continued stimulation was particularly noted.

The most recent study of electrical stimulation to luminescence is by E. B. Harvey (1917), who wrote: "When a constant current is passed through a mass of *Noctilucae*, the animals flash brightly at the make, continue glowing during the passage of the current, and cease to glow at the break, giving no flash, but sometimes they stay glowing after the break, and in this case the stronger the current the longer the glow lasts. If stimulated mechanically while the current is passing, they respond by a flash, just as when no current is passing.

"The light comes from all parts of the *Noctiluca* and is not restricted to anode or cathode regions. No increase in luminosity could be observed on the cathode side nor decrease on the anode side of the animal comparable with the polar effects of the current on muscle. . . .

"If subjected to an interrupted induced current for 45 seconds, the animals flash on the first shock and then remain glowing, but the luminosity becomes gradually fainter. If the current is now stopped for a moment and then passed again, there is again a bright glow. The animals therefore fatigue readily when stimulated electrically, as they also do with mechanical stimulation.

"If a number of *noctilucas* are punctured with a needle, causing the cells to collapse, and are then subjected to an interrupted current, they respond just as uninjured cells do. Such punctured and collapsed

cells likewise give a normal response when stimulated mechanically. . . . It is thus shown that injury to a *Noctiluca* does not interfere with its response to mechanical or electrical stimulation.

"If, however, the injury to the cells is too great, and the cells are completely broken to pieces, they do not respond to stimulation. By pressing a mass of *Noctilucae* through cheese-cloth, a filtrate was obtained containing many empty membranes and fragments of cells, visible under the microscope. This filtrate, although luminous, did not respond to electrical stimulation. . . . It is possible, however, that the fact that the *Noctiluca* juice is acid may have some effect on the response in these cases." These findings should be repeated in a well buffered medium.

It would appear from the above experiments that a complete cell membrane is not necessary for excitation of luminescence, and it would be of interest to know just how large a fragment will respond. *Noctiluca* is of great value for many lines of future work such as the time-intensity relations for excitation, all or none response, propagation of an excited state within the intact or fragmental cell or possibly from one animal to another. Nothing is known of the part played by acetylcholine in stimulation of luminescence or even of the existence of cholinesterase or other enzymes in these cells.

Salt Effects. The effect on luminescence of variations of the concentration of salt on the medium, with and without a change in the osmotic pressure, the necessity for a balanced salt content and the action of acids and alkalis have been studied by E. B. Harvey (1917). In sea water diluted with an equal volume of fresh water *Noctilucae* live for seven days, but the luminescence response to stimulation is not as bright as in sea water. In 40 per cent sea water some animals give a constant glow at first but many survive, whereas in 30 per cent sea water the glow becomes continuous and they die.

It is not the low salt content which is injurious, but the bursting of the cell by absorption of water, since *Noctilucae* in 9 parts *m* cane sugar solution and 3 parts sea water luminesce normally for an indefinite time. In pure *m* cane sugar there is normal response to stimulation for an hour and then the steady glow sets in with subsequent death, but if the cane sugar solution contains only 10 per cent sea water, the response remains normal for two days but not indefinitely. A small amount of salt appears to be necessary for continued vitality.

Certain proportions of the salts of sea water are also necessary. In pure *m* 2 NaCl, KCl, or MgCl₂ the *Noctilucae* remain normal for one or two hours, but in *m* 2 CaCl₂¹⁰ they immediately exhibit a constant

¹⁰ Possibly this salt was acid.

glow and die. A momentary constant glow may appear in pure NaCl and $MgCl_2$ solutions, but there is no rhythmic flashing, comparable to the rhythmic contraction of skeletal muscle in pure salt solutions. If Ca or Mg is added to pure NaCl in the proportions found in sea water, the behavior of *Noctiluca* is normal for longer than 24 hours, and the more similar the salt proportions to those of sea water, the more normal is the behavior of the *Noctilucae*.

As observed by many previous workers, the effect of acids and alkalis is to elicit the constant glow, with subsequent death. Concentrations of acids below the lethal value may produce a constant glow at first and later normal flashing on stimulation. It was frequently determined that a dish of *Noctilucae* plus acid that showed a constant glow would also give a flash on mechanical stimulation, but it was not determined whether the same individuals which were glowing could also flash, or whether the flash came from those more resistant individuals which were not in a state of constant glow. Future work must settle this point.

Alkalies, such as the lipid-insoluble NaOH and the lipid-soluble NH_4OH , have an effect on luminescence similar to that of acids, except that the constant glow is fainter and less lasting. In some experiments, *Noctilucae* were stained red in neutral red, which is harmless and acts as an acid-base indicator, turning yellow in alkaline solutions. The red stained *Noctilucae* changed to yellow in NH_4OH before the constant glow had ceased, indicating rapid penetration. In NaOH the color change occurred only after all luminescence had ceased and the animals were dead. This behavior is similar to that observed with many other cells. *Noctiluca* is particularly favorable material for permeability and ion exchange studies and should be thoroughly investigated by modern methods, with careful control of hydrogen ion concentration.

Narcotics and Drug Action. One of the first careful studies of narcotics on a light-emitting cell was carried out with *Noctiluca* by Massart (1893), who gave a correct description of their reversible action. His method was to place the *Noctilucae* in a large flask of sea water, where they float at the surface, and then to soak filter paper with the volatile anesthetic which was placed in the neck of the flask for a short time and then removed. The vapors dissolved at the surface of the sea water affected the *Noctilucae*. At least four types of behavior were noted. (1) When amylene or ethyl bromide was tested, the *Noctilucae* gave an initial flash of light, then became dark. If the flask was now jarred, they luminesced more brilliantly than before, a state referred to as "hyperesthesia." This effect was reversible. (2) When aldehyde, chloroform, or bromoform was used, there was an

initial flash, then a feeble continuous luminescence and no response to jarring the flask. This effect was reversible after some time—true anesthesia. (3) With ethyl acetate, acetone, and ether there was no initial flash, but a continuing feeble glow and no response to stimulation. This condition was also called anesthesia and was reversible. (4) Amyl nitrate produced anesthesia for about three minutes, but the action was irreversible, and the *Noctilucae* succumbed. Other substances (piperidine) also killed the cells without a luminous reaction. It is very likely that the different categories listed above are determined by volatility and solubility of the anesthetic, but there is no doubt that Massart definitely demonstrated true anesthesia of this luminous organism.

A later study has been made by E. B. Harvey (1917, p. 249), who used known concentrations of chloroform, ether, ethyl alcohol, butyl alcohol, chloretone, and thymol. "The effect of the anesthetic is not to prevent light production all together but to prevent a normal response, i.e., a flashing on stimulation. In all the effective concentrations, the animals under the anesthetic produce a steady glow, so faint in some cases that it is not noticeable unless the animals are present in large number. When returned to sea water, if not left too long in the solution, the steady glow ceases and the normal response returns, this is therefore a reversible phenomenon and a true case of anesthesia. The best concentrations for anesthetizing were: $\frac{1}{3}$ saturated chloroform, where the steady glow lasted 2 hours; $m/8$ ether and $m/8$ butyl alcohol, steady glow lasting $1\frac{1}{2}$ hours; $\frac{1}{16}$ saturated thymol, lasting one hour; m ethyl alcohol, lasting 30 minutes, and $\frac{1}{4}$ to $\frac{1}{5}$ saturated chloretone, lasting 15 minutes. If returned to sea water after the period of steady glow is over, the animals gave no response, the prolonged anesthesia causing death. The tentacle motion was also stopped by the anesthetic in the same concentrations as prevented normal light-response. The effect on the tentacle was, however, much slower than the effect on light-production, but took place during the early part of the period of constant glow."

The effect of various drugs on *Noctiluca* is a practically untouched field. Krukenberg (1887) has stated that veratrin, quinine, atropin and curare first stimulated to luminescence and then "paralyzed" *Noctilucae*, but there is no careful recent work relating light intensity to concentration of drug. Temperature and pressure effects on drug action, like those worked out for luminous bacteria, are also unknown, although *Noctiluca* is an ideal organism for such studies.

Temperature. The curve relating luminescence to temperature has not been determined and only a few observations on temperature limits

have been made. Quatrefages (1850) and Krukenberg (1887) both found that Noctilucae glow continuously at high temperatures and the light disappears at about 40°C. E. B. Harvey (1917) observed that the constant glow begins at 42–43° and disappears at 48–49°. "With decrease in temperature, the animals flash more than normally until the temperature reaches 5° to 0°, when they give a constant glow. If kept only a few minutes at 0°, they will recover on warming and again give a normal response; but if kept at 0° for 15 minutes they do not recover."

Illumination. After Allman (1862) had observed that the luminescence of ctenophores was inhibited by sunlight, he determined (1872) that the inhibition was not effective for Noctiluca. Since that time the evidence has been contradictory. Henneguy (1888) claimed there was no luminescence of Noctilucae obtained near St. Nazaire, France, in the daytime in summer until they had been in a dark room for three-quarters of an hour. Massart (1893) noted a night-day rhythm in luminescence of Noctilucae at Ostend and at Wimereux when kept in continual darkness. They could be stimulated to luminescence only during night-time, not in daytime, and when kept in a room with a gas light burning continuously they again luminesced only during the night hours. On the contrary, E. N. Harvey (1926) observed no effect of daylight in suppressing the luminescence of Noctiluca at Misaki, Japan.

The precautions necessary for observing day-night rhythms of luminescence have already been mentioned. Henneguy did state that the absence of luminescence was not due to his retina "because I have taken care to remain in the dark room a certain time when I took in the Noctilucae in order to accustom myself to the darkness." Massart made no mention of dark adaptation of his eyes, but his results are explicable if he was not fully dark adapted when the observations were made. Otherwise the results of both Henneguy and Massart must be attributed to a different strain of Noctilucae.

Oxygen. Both Pring (1849) and Quatrefages (1850) came to the erroneous conclusion that oxygen was not necessary for luminescence. Quatrefages found that the light still continued in a Torricellian vacuum and in hydrogen, nitrogen, and carbon dioxide gas, but he did not realize the small amount of oxygen necessary for a visible luminescence.

Later experiments by E. B. Harvey (1917) indicate clearly that Noctiluca does require oxygen for luminescence. When hydrogen gas was passed through Noctilucae in sea water, the light resulting from stimulation gradually became fainter and disappeared during the course

of an hour. On readmitting air the normal bright flash occurred when the animals were stimulated. It was noticed that those organisms caught on the sides of the vessel by a surface film of water immediately luminesced brightly at the first admission of air. It is possible that these organisms may have been injured, and it would be important to know if the admission of oxygen to normal anoxic Noctilucae can elicit a flash of light without stimulation.

The important part played by cyanide in suppressing cell oxidations immediately raises the question of its effect on luminescence. E. B. Harvey found that in decidedly concentrated KCN added to sea water (*m* 125 to *m* 250), the Noctilucae give a normal flash on stimulation for ten to thirty minutes and then show the constant glow, an effect possibly connected with alkalinity of KCN. Normal response may last for hours in weaker concentrations. In their resistance to a cyanide effect on light production, the Noctilucae behave like many other luminous animals. No studies have been made of the effect of cyanide on oxygen consumption of Noctilucae.

Biochemistry. Since Noctiluca can be obtained in large quantities, it is excellent material for macrochemical investigation, and its large size makes it well adapted for microtechniques of various kinds. Emmerling (1909) boiled Noctilucae, previously extracted with alcohol and ether to remove fat-like material, in 25% H₂SO₄ for twenty hours and determined the amino acid content. In 100 g of ash-free Noctilucae containing 7.74 g of nitrogen, he found the following amino acids:

Lysine	0.212 g with 0.040 g N ₂	Alanine	2.40 g with 0.378 g N ₂
Arginine	1.649 " " .432 " "	Leucine	0.42 " " 0.044 " "
Histidine	3.476 " " .938 " "	Proline	4.60 " " 0.556 " "
Tyrosine	0.527 " " .041 " "	Asparaginsaur	0.17 " " 0.020 " "
Glycocoll	15.90 " " 2.956 " "		
		Total	5.405 g N ₂

Pratje (1921) has made analyses of the total fat and cholesterol content of Noctilucae, extracting the dry organisms with ether in a Soxhlet apparatus. The fresh Noctilucae were preserved with 1 per cent formalin, collected in a filter and most of the salts of sea water washed out with distilled water before drying. One liter of organisms had a dry weight of 1.5 g. There was 12 per cent total lipid, 4.13 per cent non-saponizable lipid and 0.8 per cent cholesterol in the dry material. Of the total lipid 34 per cent was non-saponizable and 0.9 per cent was cholesterol. Rosenfeld (1902) had found 0.67 per cent total fat in dry Noctilucae, but his method of drying allowed the retention of sea salt, and his fat extraction was made with an alcohol-chloroform mixture instead of ether.

There are no large easily visible oil drops in *Noctiluca*, but Pratje (1921) has figured small oil droplets scattered along the protoplasmic strands after staining red in Sudan III and believed the luminescence to be connected with oxidation of these oil drops. Pratje has also made a detailed histochemical study of the *Noctiluca* nucleus based on fixed material.

Little is known concerning the "photogenic granules" scattered through the protoplasm of *Noctiluca* from which the light emission can be seen to come. E. B. Harvey (1917) determined that in living *Noctiluca* they do not absorb neutral red or methylene blue as do other granules around the periphery of the cell. The luciferin-luciferase reaction could not be demonstrated. Because of the large size of *Noctiluca*, there is a promising field for future microchemical work in connection with the contents of the vacuolar space.

Physical Characteristics. No measurements have been made, either of the light intensity or duration of a flash of *Noctiluca* or the death glow of the animal, although such determinations are now quite practical with the new photomultiplier types of photocell. Since the light comes from a population of many individual granules within the cell, the curves would in all probability be similar to those for a fire-fly flash where a large number of independent light-emitting units are also involved. The spectral distribution of *Noctiluca* light is also unknown. It is usually described as white with a bluish, sometimes a greenish, tinge, but such statements have little value.

PORIFERA

The sponges contain at least 3,000 species. They are mostly marine forms, in which the development of luminescence might be expected, since near relatives among the Protozoa and the Coelenterates are light producing. However, most reports of luminous sponges are not too convincing. Peron (1804) is credited with observing a luminous sponge, although his statement is very general. Speaking of the marine animals brought up from the depths, he merely mentioned sponges among bryozoa, hydroids, and pennatulids as phosphorescent. Noll (1879) also claimed to have seen luminescent-free swimming larvae of the sponge, *Reniera*, one of the Gelliidae of the Demospongiae, reared in a table aquarium. Noll admitted the difficulty of connecting the luminescence which appeared from agitating the water of the aquarium with a particular organism, but felt sure that the sponge larvae were responsible and that they squeezed out a luminous material by drawing together the body wall. He described bluish specks of light and yellow green points of light but stated that copepods and

worms were also present in the aquarium. No detailed description of the sponge larvae is given, and the record may be considered very doubtful because of the other probably luminous organisms present.

Dahlgren (1916) examined sponges at Naples, Italy, and came to the conclusion that worms and protozoa were responsible for the light which the sponges emitted. Further definite evidence that luminous worms are the cause of the light of sponges comes from the investigations of Okada (1925) and Trojan (1933).

Okada observed a large luminous specimen of *Crateromorpha meyeri* dredged from the bottom of the Sagami Sea at 1,000 meters. The whole body of the sponge glowed with luminescent spots for several hours after being brought into a dark room, but each spot proved to be a small annelid belonging to the family Alciopinae. Trojan (1933) examined a sponge at Villefranche-sur-Mer, France, and found that the light was unquestionably due to small specimens of *Polycirrus aurantiacus*, a terebellid worm, which used the sponge canals for a convenient dwelling.

The author (1921) has made the following observations: "At Friday Harbor, Washington, there exists a sponge, *Grantia* sp., one to three inches long, common on logs, piles, etc., in the sea water. If rubbed, a yellowish luminescence may be observed which can be obtained from all parts of the organism. If the sponge is crushed, the luminescence is quite bright. Every individual of this kind of sponge examined showed luminescence, whereas another sponge, *Esperella* sp. ?, living on *Pecten* shells, was not luminous. A few isolated dots of light only appeared on rubbing. Sponges kept in sunlight for one half hour gave as good a luminescence as those in the dark. . . .

"The sponge could not be stimulated to luminesce electrically (interrupted induced currents) under conditions when jelly-fish (*Aequorea* or *Mitrocoma*) showed a good luminescence. Examined under the microscope, no hydroids, radiolaria, dinoflagellates or Noctilucae could be observed, but many desmids, diatoms, worms and infusoria. These particular forms are not luminous, however.

"When squeezed through cheesecloth a luminous extract of the sponge was obtained, the light coming from points of light in the extract as in the case of *Cavernularia* [a pennatulid] or medusae. Addition of fresh water or saponin causes a great increase in light just as in extracts of coelenterates. No luciferin or luciferase could be demonstrated. . . . In general characters, the extracts so closely resemble those obtained from coelenterates that I am inclined to believe the light of this species of sponge is a true luminescence." The genus *Grantia*

of the Grantiidae, belongs in the Calcarea and has many closely allied genera. The *Grantia ciliata*, now called *Sycon ciliatum*, which occurs at Woods Hole, Massachusetts, behaves in quite a different way from the above and gives no trace of light on crushing or immersion in fresh water. Further careful study of calcareous sponges for luminescence is highly desirable.

CHAPTER IV

Cnidaria

CLASSIFICATION

This phylum of mostly sessile (at least during one stage of existence) plant-like organisms, the zoophytes of earlier naturalists, contains the hydroids, sea pens, sea anemones, corals, siphonophores and jelly-fish. About 10,000 species are known. Like its companion phylum, the ctenophores or comb-jellies, the Cnidaria are built of two cell layers surrounding a coeloem which also functions as a digestive cavity. A second very apparent characteristic is their true radial symmetry, which early resulted in the name Radiata, a designation now retained for a division of the animal kingdom, contrasted with a second division, the Bilateria, with bilateral symmetry, containing all the other higher phyla of the animal kingdom.

A third characteristic of both Cnidaria and Ctenophora is the rather widespread distribution of luminous species. Many hydroids and jelly-fish, almost all the siphonophores and deep water sea pens, with some gorgonians and alcyonarians, are luminous. Only among sea anemones and corals is light production lacking. As these latter forms live in the same habitat, it is rather surprising not to find luminosity developed among them. Even in deep sea anemones and corals, luminescence is said to be lacking.

The distribution of luminous groups (in italics) is shown in the classification of T. Krumbach, as follows:

Cnidaria

Hydrozoa

Hydroida (Hydroids and Medusae)

Athecatae—Anthomedusae (11 families)

Thecaphorae—Leptomedusae (9 families)

Trachylina (Medusae)

Trachymedusae (4 families)

Narcomedusae (3 families)

Siphonophora (Siphonophores)

Calycophora (5 families)

Physophora (14 families)

Scyphozoa (Jelly-fish)

Lucernariida or Strauromedusae (2 families)

?*Carybdeida* or *Cubomedusae* (3 families)

Coronata (4 families)

Semaeostomeae (3 families)

?*Rhizostomeae* (10 families)

Anthozoa

Alcyonaria

?*Alcyonacea* (Leather corals) (9 families)

Gorgonacea (Horn corals) (12 families)

Pennatulacea (Sea pens) (14 families)

Zooantharia

Actiniaria (Sea anemones) (37 families)

Madreporaria (Stone corals) (15 families)

Zoantharia (Crust anemones) (2 families)

Antipatharia (Spiny corals) (4 families)

Ceriantharia (Cylinders) (3 families)

HYDROZOA

Among the Hydrozoa and the Scyphozoa, an alternation of generations is characteristic, a vegetative polyp or hydroid stage (trophosome) alternating with a reproductive medusoid stage (gonosome). In some forms the hydroid stage, in others the medusoid stage predominates. Among the Hydroidea both generations are about equally prominent and have given rise to two systems of classification, one of the hydroids and one of the medusae. Luminous forms are scattered in several families throughout the group. An interesting point concerns the distribution of luminescence in the life cycle. If the hydroid generation is luminous, will the medusoid generations also be luminous? The evidence seems to be against a luminescence throughout the life cycle but is not complete.

The classification of the Hydroidea according to H. Brock, showing distribution of luminous genera (in italics) is as follows:

Hydroidea

Tubulariae or Athecatae—Anthomedusae

Polyp generation

Hydridae (4 genera)

Corynidae (10 genera and others uncertain)

Pennariidae (8 genera)

Myriothelidae (Myriothela)

Milleporidae (Millepora)

Tubulariidae (4 genera and others uncertain)

Branchiocerianthidae (Branchiocerianthus)

- Clavidae (7 genera and others uncertain)
- Bougainvilliidae (6 genera and others uncertain)
- Stylasteridae (9 genera and others uncertain)
- Eudendriidae (*Eudendrium*)

Medusa generation

- Codonidae with Corynidae, Pennariidae and Tubulariidae as hydroids (*Sarsia*, *Stauridium*, *Purena*, *Sarsiella*, *Slabberia*, *Ectopleura*, *Plotocnide*, *Eucodonium*, *Hybocodon*, *Pennaria*, *Corymorpha*, *Margelopsis*, and others uncertain)
- Cladonemidae with Corynidae as hydroid (9 genera and others uncertain)
- Margelidae* with Bougainvilliidae and Clavidae as hydroids (14 genera and others uncertain, including *Lizzia*, *?Oceania*, *Rathkea*, *Clavula* = *Turris*)
- Tiaridae* with Bougainvilliidae as hydroids. (19 genera, including *Stomatoca*)
- Williidae with unknown hydroids (*Willia*, *Proboscidadactyla*, and others uncertain)

Campanulariae or Thecaphorae—Leptomedusae

Polyp generation

- Lafoeidae (6 genera and others uncertain)
- Campanulinidae (9 genera and others uncertain)
- Campanulariidae* (*Campanularia*, *Clytia*, *?Laomedea*, *Obelia*, *Gonothyrea*, and others uncertain)
- Siliculariidae (*Silicularia*, *Eucopella*)
- Syntheciidae (3 genera)
- ?Sertulariidae* (9 genera and others uncertain, including *?Sertularia*)
- Haleciidae (5 genera and others uncertain)
- ?Plumulariidae* (9 genera and others uncertain, including *?Plumularia*)
- Aglaopheniidae* (13 genera, including *Aglaophenia* and others uncertain)

Medusa generation

- ?Thaumantiidae* with Campanulinidae as hydroids (*?Thaumantias*, *?Cuvieria*, *Polyorchis*, *Melicertum*, *Cannota*, *Dipleurosoma*, *Timoides*, *Spirocodon*, *Dichotomia*, *Netocertoides*, and others uncertain)
- Laodiceidae* with Campanulinidae as hydroids. (*Laodicea*, *Ptychogena*, *Staurodiscus*, *Toxorchis*, *Melicertissa* and others uncertain)
- Mitrocomidae*, with Campanulinidae and Lafoeidae as hydroids. (*Mitrocoma* = *Halistaura*, *Tiaropsis*, *Cosmetira*, and others uncertain)
- Eucopidae*, with Siliculariidae, Campanulariidae and Campanulinidae as hydroids (12 genera, including *Obelia*, *Phialidium*, and many others uncertain)
- Aequoridae* with Campanulinidae as hydroids (*Aequorea*, *Halopsis*, *Zygodactyla* and others uncertain)

Among the Trachymedusae and the Narcomedusae of the Trachylina, the hydroid generation is minute or wanting. The medusae are mostly small and transparent, with the luminescent regions usually restricted to the margin of the umbrella. The classification of H. Brock, showing known luminous forms (in italics) is as follows:

Trachylina

Trachymedusae

- Olindiidae (*Gonionemus*, *Olindias*, *Olindiodes*, *Gossea*, *Craspedacusta*, and others uncertain)

Ptychogastridae (Ptychogastria and one uncertain)

Trachynemidae (Rhopalonema, Sminthea, Homoeonema, Tetrorchis, Pantachogon, Halicreas, Halitrephes, Botrynema, Isonema, Crossota, Aglaura, Aglantha, Amphogona, Persa, and others uncertain)

Geryonidae (*Liriope*, *Geryonia*)

Narcomedusae

Cuninidae (?*Cunina*, *Cunoctantha*, *Solmissus*, and others uncertain)

Aegimidae (Aegina, Aeginura, Solmundella, Aeginopsis, and others uncertain)

Solmaridae (Solmaris, Polycolpa, Pegantha, and others uncertain)

For convenience, our knowledge of luminescence among the Hydrozoa will be considered in three sections, hydroids, hydromedusae, and siphonophores, irrespective of the fact that a hydroid and a medusa may actually be the same species in different generations. Luminescence in the Scyphozoa, large striking medusae of which only the genus *Pelagia* has been carefully investigated, will be described later.

Hydroids

Apart from early observation that stroking the plant-like growths on rocks and piles in the sea at night would often give rise to a sparkling luminescence, the first attempts to determine which organisms were the luminous ones was made by Steward (1802), who noted the "phosphoric light" of *Sertularia pumila* and thought "some particular states of the atmosphere" were involved. Peron (1804) also saw luminous sertularians and Darwin, during the voyage of the *Beagle* in 1831-36, observed a luminous coralline, a *Clytia*-like zoophyte, near *Tierra del Fuego*. A little later, Hassel (1841) described *Laomedea gelatinosa* and *Sertularia plumosa* as luminous, while Landsborough (1842), in a not too careful study, recorded luminescence of *Laomedea* (*Campanularia*) *geniculata*, *Plumularia cristata* and several luminous bryozoans but *Sertularia pumila* gave off no light when shaken. He said, "*Laomedea geniculata* was very luminous, every cell for a few moments becoming a star; and as each polyp had a will of its own, they lighted and extinguished their little lamps, not simultaneously, but with rapid irregularity so that this running fire had a very lively appearance."

Edward Forbes also knew of the luminescence of hydroids, for he wrote to a friend, George Johnston (1847, p. 150): "The finest way of observing the phosphorescence of the hydroids is to cast them into *fresh* water in the dark. The *vesicles*, when full and fresh, give out the most vivid light. This I have observed in several species of *Sertularia*."

The first connected account of luminescence in this group has been given by Allman (1871) in a section on "Phosphorescence" of his monograph on gymnoblastic or tubularian hydroids. He stated that

Obelia dichotoma flashes pale white along branches but the free planoblasts or generative buds were not luminous, although the medusa stages (*Thaumantias*) of some unknown hydroids exhibited points of light at the margin of their umbrella. When touched the hydroid light "palpitates" but lasts only a short time and fatigue soon appears. Allman noted that hydroids become momentarily luminous when removed from sea water to air and gave a persistent bright luminescence when held over alcohol vapor at 70 F. No gymnoblastic hydroid (*Athecatae*) and only certain of the calyptoblastic hydroids (*Thecap-*

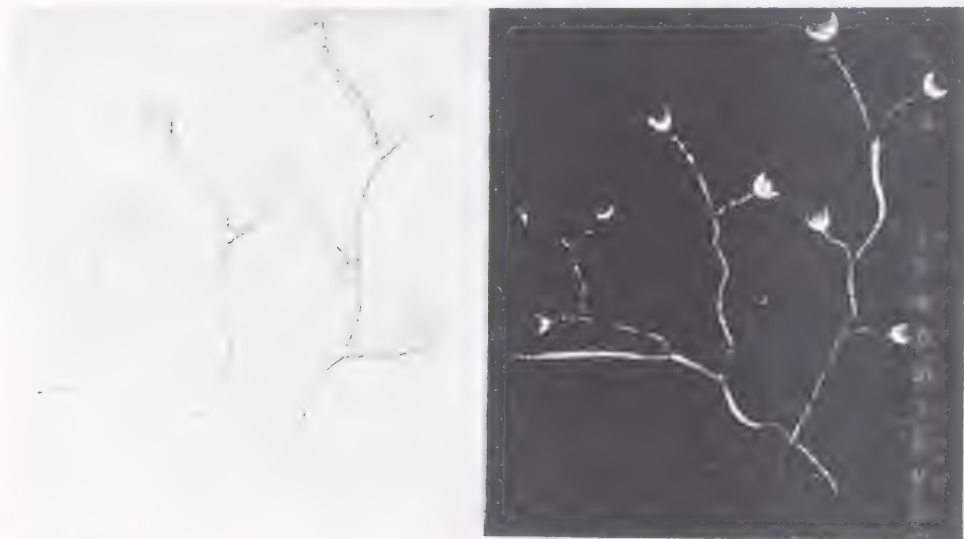


FIG. 42. Luminous hydroids, as they appear by day (left) and at night (right) After Panceri.

horae) were found to be luminescent, and there was no evidence of a secretion of luminous material. He speculated as to whether hydroid luminescence would be inhibited in the light as he (1862) had found for luminescence of ctenophores but apparently made no experiments to determine this directly.

About the same period, Paolo Panceri of Naples was engaged in his series of studies on luminous marine forms. His monograph on the Campanulariae, finished in 1876, was the last of the series, appearing in the *Atti* of the Naples Academy in 1878. Panceri described luminous *Campanularia flexuosa* growing on sea weed from the grottoes of Amalfi. His drawing, reproduced as Fig. 42, indicates that both polyp and connecting stems are luminous. Even the feet by which they adhere to rocks or plants contain luminous cells, which Panceri, by using ingenious microscopical contrivances, discovered were in the outer layer of ectoderm. They flashed only when touched but became persistently luminous in fresh water

A recent study of luminescence among the hydroids of the Woods Hole, Massachusetts, region by Sears Crowell¹ has vindicated Allman's observation that only certain species of the Thecaphorae are luminous. These are *Obelia geniculata* and *O. commisuralis*, *Campanularia* sp. and *Gonothyrea loveni*, all of the Campanularidae. *Schizotricha tenella* of the Plumularidae is not luminous. Five genera of Athecatae were examined, *Clava leptostyla*, *Hydractinea echinata*, *Podocoryne carnea*, *Pennaria tiarella*, and *Tubularia crocea*, and all were not luminous.

Crowell wrote, "In *Gonothyrea* I observed that the glow following stimulation lasted for about ten seconds and then for about three seconds the whole colony flashed on and off about ten times. Allman (1871, p. 145) reported a similar occurrence in some of the forms which he observed."

Modern knowledge of the physiology and biochemistry of hydroids is entirely lacking for they have not been carefully studied since the time of Allman and Panceri, over seventy-five years ago. It is not known whether hydroids require oxygen for luminescence, whether light has an inhibiting effect on their luminescence, whether they exhibit the luciferin-luciferase reaction or respond to addition of adenosine triphosphate. These questions can only be answered by analogy with what is known of the behavior of the medusa generation of these forms, and very few studies have been carried out on hydromedusae in recent years.

Hydromedusae

Observation that jelly-fish will luminesce goes back to Pliny's account of the "pulmo marinus," but it has not always been possible to identify the species. Pliny's marine lung was certainly the common *Pelagia noctiluca* of the Mediterranean, a Scyphomedusan, but the early descriptions by travelers of the eighteenth and early nineteenth centuries leave much to be desired. Forskal, during a trip to Egypt in 1762, Banks in 1768, Modeer in 1791, von Humboldt in 1799, Tilesius (1802),² Peron et Lesueur (1809), Macartney (1810), Macculloch (1821), Quoy et Gaimard (1825), Baird (1830), Webster³ (1834), Rathke⁴ (1835), Ehrenberg (1832), Loeffling (1838), Bennett (1837),

¹ Private communication in 1939.

² It is possible that Tilesius observed luminous bacteria growing on the medusae, as he speaks of keeping them in a cool dry place for a night and that they do not become as bright or as odorous as decaying squid.

³ In all probability Webster saw luminous ctenophores.

⁴ Rathke gives a detailed description and beautiful plate of a small medusa, *Oceania Blumenbachii*, but he did not actually determine from which part the light came.

Brandt (1838), Forbes (1848), Busk (1852), Panceri (1872), Meldola (1884), Kiernik (1908), and many others have described luminous "medusae," most of them hydromedusae, belonging to the Hydrozoa.

Among the four types of hydromedusae, the Trachymedusae and Narcomedusae, with a hydroid generation minute or lacking, contain few luminous species. The genera *Liriope*, *Geryonia* and *Solmissus* appear to be the only luminous ones. It is in the Leptomedusae and to a lesser extent in the Anthomedusae, where the hydroid generation is well developed, that luminescence is frequent, usually appearing in definite groups of cells, often yellow in color, situated at the base of the tentacles.

Forbes (1848) devoted four pages to "Phosphorescence" in his Monograph of the Naked-Eyed Medusae, giving an excellent history of previous observations on luminous forms. He observed luminous *Turris*, *Oceania*, *Dianaea*, and *Thaumantias* near the Zetland Islands and saw "*Dianaea appendiculata* radiate greenish luminescence from reproductive glands." In the Mediterranean Forbes noticed "a large *Mesonema* give out rich flashes of flame from the bases of its numerous marginal tentacles" and concluded: "It would seem that phosphorescence in the naked-eyed Medusae is developed by the reproductive and motor systems: how we cannot say." As in the case of hydroids, Forbes advocated fresh water as a medium to reveal the luminescent regions.

Histology. The first attempt to investigate the structure of the luminous regions of hydromedusae must be attributed to Busk (1852), who collected a *Thaumantias* like medusa, $\frac{3}{4}$ in. diameter, near Portsmouth, England. This form was luminescent at the bulbs of the tentacles and very bright when placed in fresh water. The bulbs contained solid glandular cells, rather poorly figured by Busk, which he thought secreted a luminous material.

The next observer was Panceri (1872), who devoted most attention to *Pelagia*, but also studied *Cunina moneta*, a narcomedusan as "clear as pure polished crystal." The light is bluish and very intense so that it can be seen in daylight on a cloudy day. Luminescence never appears on the disk but only on the rather stiff tentacles and on the membrane which hangs down from the crown of tentacles. Otherwise the behavior of the medusae is the same as that of *Pelagia*, luminescing on stimulation or when placed in fresh water: "where one can see little sparks of luminescence detach themselves from the membrane and wander about in the water." Again there were present cells filled with

* Brandt devoted 10 pages to the light of medusae, mostly filled with the observations of others.

yellow, very refrangible granulations resembling fat, which floated to the surface. The essence of light production in all these medusae was believed by Panceri to be the slow oxidation of a fatty substance like that of the dead fish he had previously described.⁶

The author (1921) studied four species of hydromedusae at Friday Harbor, Washington: *Aequorea forskalea*, *Mitrocoma* (*Halistaura*) *cellularia*,⁷ *Phialidium gregarium*, and *Stomatoca atra*. The hydroid of *Aequorea* is probably a *Campanulina*, that of *Mitrocoma* is unknown, that of *Phialidium* is *Clytia inconspicua* and that of *Stomatoca* probably a tubularian, *Perigonimus*. If *Perigonimus* turns out to be luminous, it would form an exception to the general rule that only thecate hydroids are luminous.

The light, of a bright bluish green,⁸ came from spots along the edge of the umbrella at the base of the tentacles. No other regions of *Aequorea* or *Mitrocoma* phosphoresced, but at times faint light was to be observed coming from masses (gonads) along the four radial canals of *Phialidium*. Examination with the microscope in the daytime discloses on the margin of the umbrella of *Aequorea* oval masses of yellow tissue corresponding in position with the luminous areas at night. The animal is shown in Figs. 43 and 44. In *Mitrocoma* the yellow masses are much closer together, forming an almost continuous line in some places. The light of *Stomatoca* is faint and rather diffuse.

The yellow tissue is undoubtedly the luminous tissue. Examined at night under the microscope, the yellow spots present a beautiful luminous appearance. Under conditions which cause cytolysis of the cell, such as addition of fresh water or saponin, it can be clearly seen that the light comes from large granules with a definite boundary—light discs. They are not mere points of light. The light discs vary in size and luminesce for some time, flashing out very brightly and then slowly fading.

The luminescence of hydromedusae is probably to be considered extracellular. On merely touching these jelly fish one cannot observe that any luminous secretion is thrown into the water, as in the case of the ostracod, *Cypridina*, but on very gentle stroking of the edge of the umbrella a mass of luminous material comes off which adheres to the fingers, or on tossing an animal on the surface of the water, abundant luminous material is liberated which causes the sea water to luminesce. Luminous material accompanies the slime so commonly secreted by

⁶ The fish luminescence was undoubtedly due to luminous bacteria.

⁷ This medusa is sometimes called *Thaumantias cellularia*.

⁸ Examination with a hand spectroscope disclosed a band extending approximately from $\lambda = 0.46-0.60\mu$ with limits nearly the same for both *Aequorea* and *Mitrocoma*.

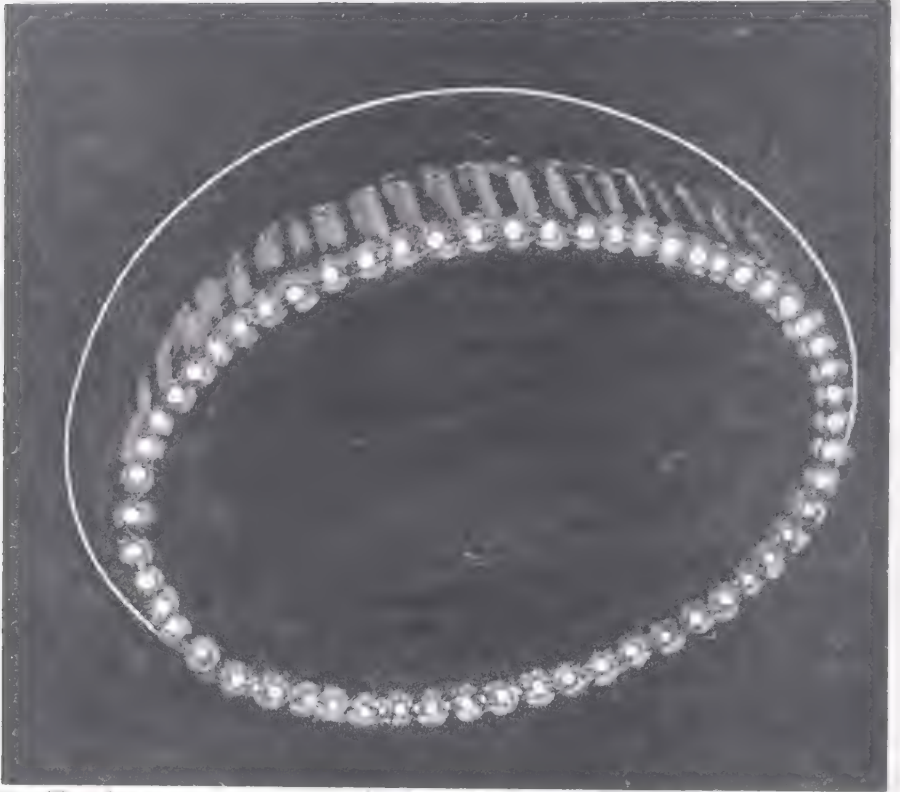


FIG. 43. *Aequorea aequorea*, showing the regions of luminous tissue. From M. E. Johnson and H. J. Snook, *Seaside Animals of the Pacific Coast*. Copyright 1927, reproduced by permission of The Macmillan Co

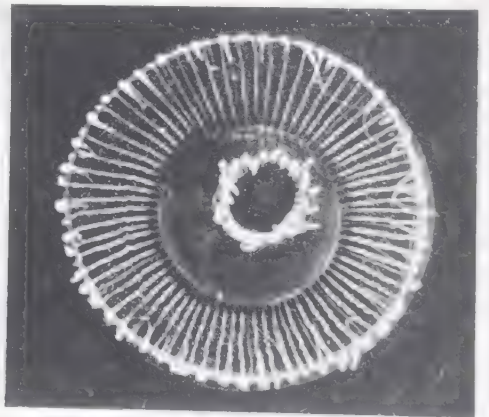
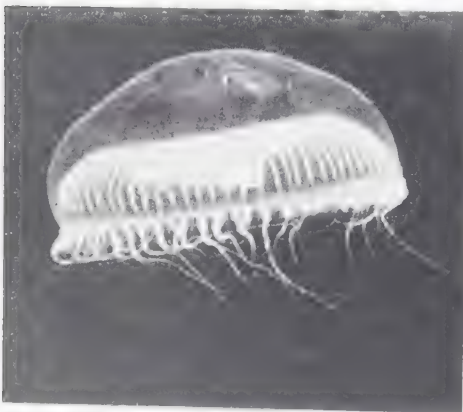


FIG. 44. Photograph of *Aequorea aequorea* by day, side (left) and oral (right) view. From M. E. Johnson and H. J. Snook, *Seaside Animals of the Pacific Coast*. Copyright 1927, reproduced by permission of The Macmillan Co

these organisms. Apparently the luminous cells are easily ruptured, with discharge of their contents into sea water. No modern description of the histology of hydromedusae has been made, and the fine structure of the luminous spots is completely unknown.

Physiology. Modern studies on the physiology of hydromedusae are lacking. It was early recognized that the light only appeared as a result of stimulation, and von Humboldt (1822) was apparently the first to discover that luminescence could be elicited by electrical stimulation. During his journey to the Equinoctial Regions of the New Continent during the years 1799–1804, numerous medusae were collected between Madeira and Teneriffe in the Canaries. He wrote, "If we place a very irritable medusa on a pewter plate, and strike against the plate with any sort of metal, the small vibrations of the plate are sufficient to make this animal emit light. Sometimes in galvanizing the medusa, the phosphorescence appeared at the moment the chain closes, though the exciters are not in immediate contact with the organs of the animal." There is no detail regarding the electrical apparatus he used, but it was no doubt similar to that used for his previous nerve and muscle studies, which were made in 1797, soon after Galvani's famous frog nerve discoveries.

Macartney (1810) also studied mechanical and electrical stimulation of a form which he called *Medusa hemispherica*, evidently a hydromedusan judging by his description: "Some hemispherical medusae were placed in contact with the two ends of an interrupted chain, and slight electric shocks passed through them. During the very moment of their receiving the shock no light was visible, but immediately afterwards the medusae shone like illuminated wheels, which appearance remained for some seconds. Upon the closest inspection with a magnifying glass, no contractile motion could be perceived to accompany the exhibition of the light. The application of electricity in this instance seems to have acted merely as a strong mechanic shock." Macartney also found that when heated or when plunged into "spirits," the luminescent spots again appeared "like illuminated wheels." He placed some medusae in a vacuum and could not discover that the light was any less brilliant, in fact it was more easily excited by shaking and continued longer in a vacuum.

Biochemistry. A few experiments on the chemistry of luminescence in hydromedusae have been carried out by the author (1921). Luminous extracts were prepared from strips of the margin of the umbrella of *Aequorea* or *Mitrocoma*, cut off with scissors. When squeezed through four layers of cheesecloth, these strips become a luminescent solution which glows for some hours, in one case for nine hours.

When the luminescence disappears, the addition of fresh water, gentle heating, cytolytic agents such as saponin, sodium glycocholate, chloroform, ether, or NaCl crystals will again revive the luminescence. However, once the light has disappeared by addition of saponin or Na glycocholate powder, fresh water will cause no more light to appear.

Isotonic cane sugar solution or sea water does not cause emission of light from these extracts. The phenomenon appears to be one of cytolysis and granulolysis—by diminution of osmotic pressure, by heat, or by addition of specific substances. It is probable that in the extract intact photogenic cells and also photogenic granules are present and dissolve with production of light. Microscopic observation at night reveals the sudden appearance of a disc of light, too small to be the illumination of a cell, but capable of interpretation as the light from an isolated granule.

Attempts to demonstrate luciferin and luciferase have failed. Extracts of *Aequorea*, which should contain luciferase, give no light with boiled extracts of *Aequorea* which should contain luciferin. The same is true for *Mitrocoma* and for crosses of luciferin and luciferase of *Cypridina* with "luciferin" and "luciferase" of these two medusae. Like many other luminous animals the luminous material of *Aequorea*, *Mitrocoma*, or *Pholidium* can be dried over CaCl_2 and will give a bright light when again moistened.

It will be recalled that Macartney (1810) found no diminution of light when he placed medusa *hemisphaerica* in a vacuum. Such behavior might be questioned were it not for more modern observations on radiolarians, ctenophores, and *Pelagia* (Harvey, 1926), which indicate the ability to luminesce in absence of oxygen. R. S. Anderson⁹ has tested *Aequorea* at Friday Harbor and found that this hydro-medusan will also luminesce under strict anaerobic conditions.

Finally the author has tested the ability of all four species of medusae—*Aequorea*, *Mitrocoma*, *Phialidium*, and *Stomatoca*—to luminesce in bright daylight. Immediately after collecting in the morning they were brought by an assistant to a dark room where it was found that the luminescence was as bright as at night. There is no inhibition by light and no day night rhythm of luminescence in these forms.

Siphonophora

Probably less is known of the luminescence of these floating colonies of Hydrozoa than of any other of the coelenterates. Although *Physalia*

⁹ Private communication in 1939.

¹⁰ M. E. Johnson and H. J. Snook in *Seaside Animals of the Pacific Coast*, 1927, p. 69, State that *Aequorea*, *Halistaura*, *Phialidium*, and the ctenophores, *Bolinopsis* and *Pleurobrachia* will not luminesce in daytime unless left in the dark for an hour.

was reported as luminous by Tilesius (1819), the identification is questionable and the present genus *Physalia*, the Portuguese man-of-war, is not luminous. It is also quite certain that the related *Velella* and *Porpita* are also non-luminous. Light emission in this group is confined to the delicate transparent forms and appears to have been first reported by Meyen (1834) for *Diphyes*, shown in Fig. 45.

Peach (1850) also found the *Diphyidae* responsible for some of the sea light along the English coast. Giglioli (1870) listed *Diphyes*, *Praya*, *Abyla*, and *Eudoxia*, all from the high seas, and Panceri (1871) *Praya cymbiformis* and *Abyla pentagona* in the Bay of Naples as luminous forms. The light is usually described as rather faint, a description

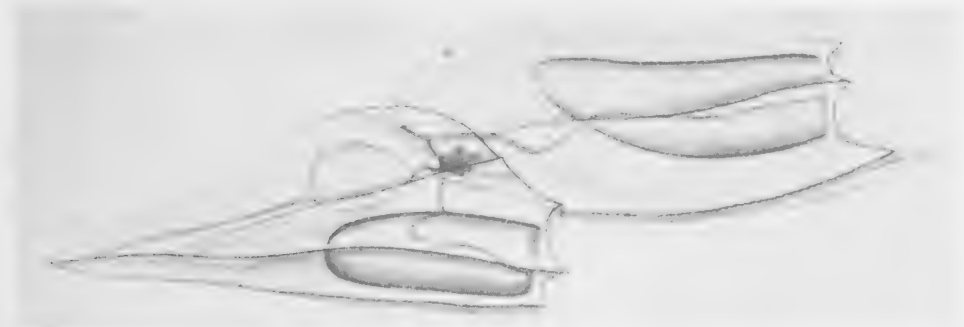


FIG. 45. The siphonophore, *Diphyes*. After Bigelow.

certainly true of a form, *Algamopsis* (*Algamia*) *elegans*, which the author caught in August, 1921, in the cold waters at the mouth of the Bay of Fundy near St. Andrews, New Brunswick.

Dubois (1914, p. 42) has stated that many varieties of siphonophores are luminous and emphasized particularly the remarkable behavior of *Hippopodius gleba*, common at Villefranche on the Mediterranean. The colony is transparent at rest but on stimulation becomes milky and opalescent, "at the same time developing a magnificent bluish illumination of the surface." Examined with a microscope Dubois observed the epithelial cells alone to become clouded from formation of a multitude of granulations caused by shaking, just like the sudden appearance of crystals in a supersaturated solution when given a sudden blow. Dubois held that the appearance of light and the appearance of these granulations were definitely connected, a circumstance similar to that he had previously observed with *Noctiluca*. It would seem that this "clouding" should be reinvestigated by modern optical methods now available, especially since no histological, physiological, or biochemical studies on luminous siphonophores have been made. The position of the known luminous genera (in *italics*) in the classification of F. Moser can be seen from the following list.

Siphonophora

Calycophora

Monophyidae (Sphaeronectes, Monophyes, Muggiaea, Nectopyramis, Heteropyramis)

Diphyidae (Galeolaria, *Diphyes*, Ceratocymba, *Abyla* = *Abylopsis*, Bassia, *Praya*, Lilyopsis, Nectodroma)

Dimorphyidae (Dimophyes, Amphicaryon, Mitrophyes, Cuboides)

Polyphyidae (*Hippopodius*)

Stephanophyidae (Stephanophyes)

Physophora

Apolemiidae (Apolemia)

Forskaliidae (Forskalia, Erenna)

Agalmidae (*Agalma*, Halistemma, Anthemodes, Lychnagalma, Stephanomia)

Pyrostrephidae (Pyrostephos)

Nectaliidae (Nectalia)

Physophoridae (Physophora)

Athoriidae (Athoria)

Anthophysidae (Athorybia, Anthophysa)

Rhodaliidae or Aurnectidae Angelopsis, Archangelopsis, Dromalia, Rhodalia, Steleophysema)

Rhizophysidae (Rhizophysa, Bathyphysa = Pterophysa)

Epibuliidae (Epibulia)

Physaliidae (Physalia)

Velellidae (Velella)

Porpitidae (Porpita, Porpema)

SCYPHOZOA

Introduction

All the great naturalists of the sixteenth and seventeenth centuries —Rondelet, Boussuet, Aldrovandi, Belon, Gesner, Kircher— speak of the luminous "Pulmo marinus," referring to *Pelagia noctiluca*, and repeat Pliny's story of the slime from this form which adheres to fingers and covers objects with a luminous coat. Although some other Scyphomedusae have been reported as luminous, the entire knowledge of luminescence among Scyphozoa is derived from this genus,¹¹ a member of which is shown in Fig. 46. The position of luminous genera (in italics) is shown in the classification of T. Krumbach, as follows:

Scyphozoa

Lucernariida or Stauromedusae

Eleutherocarpidae (8 genera)

Cleistocarpidae (4 genera)

¹¹ In Mayor's *Medusae of the World* seven species are recognized. *Pelagia noctiluca* and *Pelagia phosphorea* are luminous, but nothing is known of light production of the others.

Carybdeida or *Cubomedusae**?Carybdeidae* (*?Carybdea* or *Tamoya*, *Tripedalia*)*Drepanochiridae* (*Drepanochirus*, *Chiropsalmus*)*Chirodropidae* (*Chirodropus*)*Coronata**Periphyllidae* (6 genera)*Ephyropsidae* (3 genera)*Atorellidae* (*Atorella*)*Atollidae* or *Collaspidae* (*Atolla*)*Semaeostomeae**Pelagidae* (*Pelagia*, *?Chrysaora*, *Dactylometra*, *Kuragea*, *Sanderia*)*Cyaneidae* (*Cyanea*, *Drymonema*, *Patera*)*Ulmaridae* (10 genera including *Aurelia*)*Rhizostomeae**Cassiopeidae* (*Cassiopea*)*Cepheidae* (3 genera)*Mastigiadidae* (3 genera)*Versuridae* (*Versura*)*Leptobranchidae* (*Thysanostoma*, *Lorifera*, perhaps others)*Lychnorhizidae* (*Lychnorhiza*, *Pseudorhiza*)*Catostylidae* (6 genera)*Lobonemidae* (*Lobonema*, *Lobonemoides*)*?Rhizostomidae* (*?Rhizostoma*, *Rhopilema*)*Stomolophidae* (*Stomolophus*)

FIG. 46. *Pelagia noctiluca*, a luminous jelly-fish common in the Mediterranean. After Steuer.

Scientific study of *Pelagia* luminescence begins with the Abbe Spallanzani's observations, made at Messina, Sicily, in 1788. After a detailed description of the anatomy and a study of the pulsation, Spallanzani directed his attention to the relation between luminescence

and movement. He had noticed that the light was much brighter during systole than diastole and had previously observed luminescence in the worm, *Nereis marina*, when it moved, and lighting of the glow-worm with each oscillation of the body. He therefore concluded it was probable that the increase in brightness on systole resulted from a stimulation of some kind which set off pulsation and luminescence simultaneously. Many observers since then, for example Quatrefages in 1850 and Watasé as late as 1898, have called attention to movement and luminescence. However, this relationship is not fundamental, for an undisturbed *Pelagia*, swimming quietly through calm water does not spontaneously luminesce with every pulsation.

Taking medusae to his house where they were kept in large glass vessels, Spallanzani noted that the light continued after death, when decomposition had set in, although it was weaker. To his astonishment, a medusa that had remained on a sheet of paper for 22 hours and which had been mostly liquefied and was completely dark, became very bright on adding fresh water (but not sea water), bright enough to read print. Spallanzani referred to these liquefied medusae as dried medusae and has been credited with observing that luminous animals could be dried and would again luminesce on moistening, but it is a question as to how dry they really were, especially since he found that movement, as by rubbing the "dried" dark medusae with his finger would revive the light again. The luminous material was a mucus formed on the edge of the umbrella and especially abundant on the arms. The mucus could be made to light on mixing with water, urine, or milk, and the rest of the animal, devoid of this mucus, could not be made to light in any way. The luminescence in milk was so great "that I could read the writing of a letter at three feet distance."

Nearly a century elapsed before a more modern study of *Pelagia* was undertaken by Panceri (1872), who again called attention to the luminous slime formed after stimulation of various kinds, including the electric pile, the effect of fresh water and milk and elevation of temperature. Sea water at the freezing point did not extinguish the light nor did the medusae luminesce differently in oxygen or carbon dioxide than they did in air. Light did not inhibit the luminescence, and the spectrum was monochromatic, a pale greenish band.

Histology

In the mucus of *Pelagia* under the microscope, Panceri (1872) observed both nettle cells and epithelial cells, many of which were full of fine yellow granules which had every appearance of being fat. These granules were undoubtedly the source of the light, and Panceri believed

that the epithelium was continued into the canals and was responsible for the luminescence observed in them and surrounding the edge of the ovaries.

A study of sections of the umbrella has been made by Dahlgren (1916), who observed on the outer surface large "luciferine"-producing cells with large granules, smaller mucus-producing cells, some vacuolated cells which had presumably emptied their secretion, and a few other types, as shown in Fig. 47. Dahlgren noted that if the exumbrella surface was stroked with a finger which was immediately with-

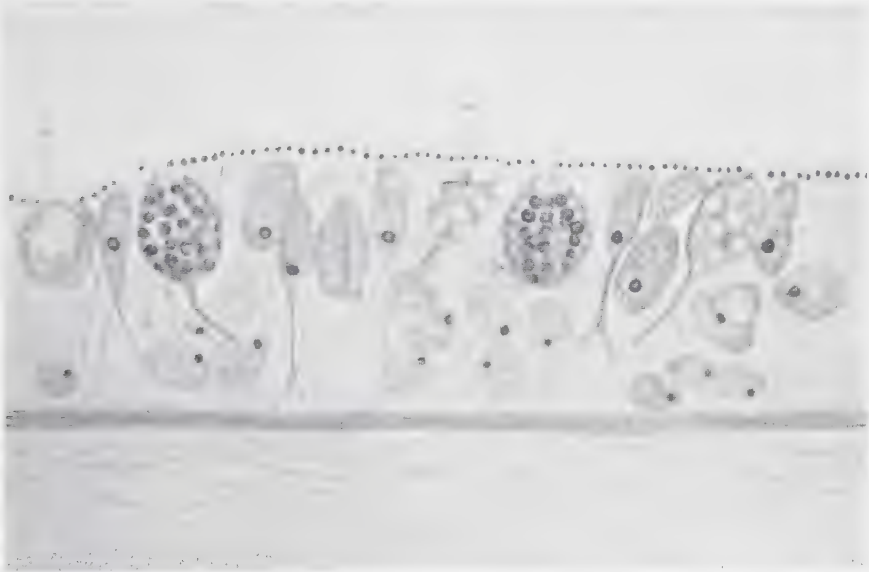


FIG. 47. Section of the aboral umbrella surface of *Pelagia noctiluca*, showing l, luminous cells, m, mucous cells, and v, cells with contents discharged. After Dahlgren.

drawn, no luminous material adhered to the finger; only if the stroking was slow or the surface was scraped did luminous mucus come off, leaving the tissue underneath non-luminous. There can be no doubt of an external secretion of luminous material in this form, but only if the mechanical disturbance is considerable.

Physiology

Physiological studies on *Pelagia* have been largely carried out by Heymans and Moore (1923, 24) and by Moore (1926). Their work has been concerned with the effect of various ions on light production and stimulation by the electric current, believed to be closely connected with ion action.

Electrical Stimulation. Although Panceri (1872) observed that a

glow would move over the disk of *Pelagia* from a touch at one point, the spread of light is not as marked as in pennatulids, where a stimulus to one part of the colony produces several waves of light which pass from animal to animal over the whole colony. In *Pennatula* the excitation is carried by nerves at a rate generally characteristic of impulses in motor nerves in coelenterates.

In *Pelagia* excited by a constant current, Moore has described a glow which occurs along the margin on the anodal side of the animal when a current of 200 milliamperes was passed through.¹² "In very sensitive specimens the luminescence spreads from this region like a blush over the whole bell. The glow continues during the flow of the current and ceases at the break."

Observation of a glow at the anode on the make was rather surprising since Pflüger's general law of stimulation states that excitation should occur at the cathode when a direct current is made. The usual explanation of this effect attributes cathodal excitation to increased irritability at the cathode where an excess of Na ions collects. This reversal of Pflüger's law required further study since Moore observed the same phenomenon of luminescence at an anode when the ctenophores, *Beroë* and *Mnemiopsis*, were subjected to direct currents.

To test the question as to whether the current acted directly on luminous cells and photogenic material or on nerve fibers which in turn stimulated the luminous cells, some of the luminescent slime was collected in a watch glass, and the current was passed through. A bright glow occurred at the cathode on the make and during passage of the current, but this cathodal luminescence proved to be due largely to the alkali which is formed at the cathode. Since no anodal luminescence appeared in the slime, the anodal luminescence of whole medusae was attributed to excitation of nerve elements which in turn stimulated luminous gland cells. These elements occur only along the margin of the bell, for if this region is cut off, no luminescence of the remainder of the bell can be obtained.

The reversal of Pflüger's law does not appear to be a phenomenon connected specifically with direct excitation of luminescence. It has in fact been observed in stimulation of other tissues and may be of more widespread occurrence than generally supposed.

Ion Effects. Analysis of the behavior of *Pelagia* in solutions of different salt content is complicated, since the effects may involve sensory endings, nerves, pulsation centers, muscles, or luminous gland cells. The motor behavior of this medusa is similar to that of medusae in general. In the luminescence response it is necessary to distinguish be-

¹² Moore used platinum electrodes but stated that the same results are obtained with non-polarizable electrodes.

tween the local luminescence due to direct stimulation of gland cells and the general luminescence from strong stimulation, involving the whole bell and tentacles.

The general effects on the whole medusa of altering the ion content of the sea water has been described by Heymans and Moore (1924) as follows: "1. In a solution from which CaCl_2 is omitted the pulsations of *Pelagia* stop in 3 to 5 minutes. At the same time general luminescence fails, so that when the animal is strongly stimulated light appears only in the area of contact. If now the proper amount of CaCl_2 be added to the solution or if the animal be returned to artificial sea water, the beats reappear within 90 seconds and simultaneously the power of general luminescence is restored. It must, therefore, be concluded that CaCl_2 is necessary to the conduction of impulses both for the muscular beats and for general luminescence.

"2. If the solution lacks K ions, after 10 minutes the pulsations stop in the diastolic phase and at the same time the power of general luminescence is lost. When the animal is replaced in sea water or in complete van't Hoff's solution the beats return within 65 seconds and general luminescence is reestablished in 140 seconds. These results prove that K like Ca is necessary for normal beats and for the conduction of the impulse for general luminescence.

"3. In a solution containing no Mg salts, *Pelagia* shows great acceleration of beat, and after 11 minutes stops in systole with spasmodic fibrillation. During this time automatic flashes of light appear and, if the body is touched, the whole surface breaks into light and glows for some seconds. This condition persisted for at least an hour and a quarter, at the end of which time the observations were discontinued. Since the absence of Mg results in a condition of hyperirritability both with regard to rhythmical contractions and general luminescence, it follows that Mg ions must act to decrease irritability. The locus of the action of Mg on the luminescence reaction is some part of the nervous system, since an excess of Mg inhibits general luminescence, but does not affect local luminescence."

In CaCl_2 poisoning, according to Moore, *Pelagia* becomes hypersensitive. Spontaneous luminescence spreads over the whole bell and the muscles contract. Under these conditions luminescence appears to be connected with contraction, but the two functions are quite distinct. In a solution of KCl, the musculature of *Pelagia* is completely relaxed while luminescence appears throughout the bell and tentacles. Furthermore, during passage of a constant current, the rhythmic contraction and relaxation of the musculature occurs at a time when the animal glows continuously.

In order to test the effect of salts on the luminescent material

directly. Moore collected the slime made up of the contents of luminous gland cells, on filter paper. Normally this "indicator paper" remains dark when torn or rubbed or when placed in sea water or 1.1 *m* cane sugar, isosmotic with sea water of Naples Bay. However, in certain pure neutral isosmotic (0.6 *m*) salt solutions, luminescence appears brightest in the following series: MgSO_4 , K_2SO_4 , Na citrate, KCl, BaCl_2 , CaCl_2 , and LiCl. In NaCl and MgCl_2 there is no light, suggesting that these salts are present in sea water in sufficient concentration to inhibit the luminescent reaction. Moore found that alkalis, particularly NH_4OH , even as strong as 0.9 *m* concentration, in isosmotic cane sugar cause luminescence of the filter strips while mineral acid inhibits the stimulating action of MgSO_4 and KCl.

Temperature has a marked effect on the luminescence. The duration of glow of filter strips placed in 0.6 *m* MgSO_4 solution or in 0.9 *m* ammonia in isotonic cane sugar varies from 5 to 10 minutes at low temperatures ($5-10^\circ$) to a few seconds at high temperatures ($40-50^\circ$). The temperature coefficients (Q_{10}) average around 2. Raising the temperature of sea water containing "indicator paper" does not itself cause luminescence. Although certain aspects of the behavior of the "indicator paper" suggest that osmotic cytolysis of cells occurs, the behavior in pure salt solutions and the negative effect of rise of temperature show that something more than ordinary cytolysis is involved.

Later studies on salt effects were made in a short note by Hykes (1928), who used four species of medusae, including *Pelagia noctiluca* and *Aequorea forskalea*. Action of various pure salt solutions was noted on both movement and luminescence of whole animals. Hykes reported that pure isotonic NaCl has little effect on luminescence, perhaps making the medusae slightly more luminescent, and that the intensity is greater in those individuals kept in NaCl in the dark than those in the light. In isotonic KCl, luminescence increases, becoming especially bright on stimulation and often spontaneous (autonomous). In isotonic MgCl_2 , at a time when narcosis of muscular contraction has occurred spontaneous luminescence could still appear. Later this autonomous luminescence disappears but the whole medusa can still luminesce when touched. In isotonic CaCl_2 , luminescence is not diminished, but on the contrary very intense, even after six hours, when a slight blow on the container provoke a strong luminescence even when the medusa is beginning to disintegrate. In general, Hykes was impressed with the persistence of the ability to luminesce for a long time after other vital manifestations had ceased.

Effect of Illumination. Panceri (1872) observed no inhibitory effect of light on the luminescence of *Pelagia* but Heymans and Moore

(1923, 24) stated that this medusa showed a diurnal rhythm in luminescence, lighting on mechanical stimulation only at night. They also studied at night the inhibiting effect of a carbon arc, which affected the general luminescence but not the local luminescence. "These results suggest that strong illumination acts on the nervous mechanism to inhibit general luminescence but exercises no direct effect on the luminescent organs themselves since local luminescence persists. It should be noted, however, that during the day excitation of *Pelagia* fails to elicit local luminescence even after five hours in the dark room." A study of the suppression of luminescence by the carbon arc indicated that suppression depends on time \times intensity, i.e., on the quantity of light or the exposure.

Curiously enough, Moore (1926) was unable to confirm the day-night rhythm or the inhibiting effect of light when studied two years later. "Even exposure to sunlight for half an hour did not appreciably reduce the luminescence which appeared on stimulation of the animal in the dark. I have no idea how to account for such an extraordinary difference in behavior during the two seasons." The author (1926) has been unable to observe luminescence inhibitory effects of light on *Pelagia* luminous material. Ries and Ries-Imchanitzkey (1940) have compared the luminescence of *Pelagia* to a radium paint which cannot be extinguished by exposure to red light, but such a comparison has little meaning.

Biochemistry

Like the *Radiolaria* and *Ctenophora*, *Pelagia noctiluca* does not require oxygen for luminescence. As shown by the author (1926) an extract of *Pelagia* rendered free of oxygen by adding sodium hydro-sulfite, or by hydrogen and platinized asbestos, will luminesce brightly on mixing (in a pure hydrogen atmosphere) with distilled water, also freed of oxygen by the above methods. During the whole time of mixing, methylene blue, which was used to detect traces of oxygen remained completely reduced.

Despite many attempts it has not been possible to demonstrate a luciferin-luciferase reaction with *Pelagia* nor will extracts of *Pelagia* so prepared that they should contain luciferin luminesce when mixed with *Cypridina* luciferase. The opposite mixture, *Cypridina* luciferin and *Pelagia* luciferase, is also negative. Finally, examination of *Pelagia* in near ultraviolet light (without the visible) revealed no unusual fluorescence of the regions of the animal containing the luminescence gland cells (Harvey, 1926).

ANTHOZOA

None of the stone corals or the sea anemones (the Zooantharia) have thus far been reported as luminous, but among the Alcyonaria are to be found some of the most brilliant and striking luminous animals. Of the three groups of Alcyonaria, the Alcyonacea, the Gorgonacea, and the Pennatulacea, only luminescence of the Pennatulacea has been carefully studied and will be considered in a special section. According to the classification of W. Kükenthal, the relationship of the various groups with luminous genera is indicated in the following table, but it is probable that many more contain luminous species than are indicated by italics.

Anthozoa

Octocorallina or Alcyonaria

Alcyonacea

- Cornulariidae (Cornularia, Anthelia, Clavularia, Acrossota)
- Tubiporidae (Tubipora)
- Telestidae (4 genera)
- Xeniidae (Xenia, Cespitularia, Sympodium)
- ?*Alcyoniidae* (9 genera, including ?*Alcyonium*)
- Nephthyidae (12 genera)
- Siphonogorgiidae (Nephthyigorgia, Siphonogorgia)
- Fasciculariidae (Fascicularia, Paralcyonium, Studeriotes)
- Helioporidae (Heliopora)

Gorgonacea

- Briareidae (14 genera)
- Suberogorgiidae (Suberogorgia, Keroeides)
- Coralliidae (Corallium, Pleurocoralloides)
- Melitodidae (6 genera)
- Plexauridae (12 genera)
- Acanthogorgiidae (Acalycigorgia, Acanthogorgia)
- Muriceidae (27 genera)
- Primnoidae (12 genera)
- Gorgoniidae (11 genera, including Gorgonia)
- Gorgonellidae (6 genera)
- Chrysogorgiidae (7 genera)
- Isididae* (*Isidella*, *Lepidisis*, *Acanella*, *Ceratoisis*, *Peltastisis*, *Primnoisis*, ?*Mopsea*, *Muricellisis*, ?*Isis*, *Chelidonisis*)

Pennatulacea

- Veretillidae* (*Lituaria*, *Cavernularia*, *Veretillum*)
- Echinoptilidae (Echinoptilum, Actinoptilum)
- Renillidae* (*Renilla*)
- Kophobelemnidae (Schlerobelemnon, Kophobelemnion)
- Anthoptilidae (Anthoptilum)
- Funiculinidae* (*Funiculina*)
- Protoptilidae (Protoptilum, Distichoptilum)
- Stachyptilidae (Stachyptilum)
- Scleroptilidae (Scleroptilum, Calibelemnon)

Chunellidae (*Chunella*, *Amphiacme*)

Umbellulidae (*Umbellula*)

Virgulariidae (*Pavonaria*, *Virgularia*, *Styatula*)

Pennatulidae (*Acanthoptilum*, *Scytalium*, *Pennatula*, *Leioptilus* = *Ptylosarcus*)

Pteroeididae (*Pteroeides*, *Sarcophyllum*, *Gyrophyllum*)

Hexacorallina or Zooantharia (5 orders and 61 families, not luminescent)

Alcyonacea and Gorgonacea

Concerning the Alcyonacea and Gorgonacea little is known except reports of luminescence by early explorers. Peron (1804) mentioned "gorgones," together with "de retepores, de sertularies, d'isis, d'alcyons et d'éponges," as being luminous. Carpenter, Jeffreys, and Thomson (1869) speaking of the results of dredging by the *Porcupine* in 1861 said the Pennatulæ, the Virgulariæ, and Gorgoniæ "shone with a lambent white light, so bright that it showed quite distinctly the hours on a watch. *Pavonaria quadrangularis* was pale lilac like the flame of cyanogen while that from *Ophiocantha spinulosa* [a brittle star] was of a brilliant green. . . ." Sir Wyville Thompson (1872) in *Depths of the Sea* and later in his accounts of the Voyage of the *Challenger* (1877) during the years 1873-76 constantly remarked (Vol. I, p. 119) on the pale lilac phosphorescence of slender gorgonians. In fact Moseley (1877), who made a study of the coloring matter and the luminescence of animals collected by the *Challenger*, especially the deep sea forms, wrote: "All the Alcyonarians dredged by the *Challenger* in deep water were found to be brilliantly phosphorescent when brought to the surface and their phosphorescence was found to agree in the manner of exhibition with the same conditions as are observed in the case of shallow water relatives."

The Marquis De Folin also, in his book, *Sous les mers*, published in 1887 (pp. 131-132), an account of the French explorations in the *Trauvaille* and *Talisman* in 1880-83, mentioned among other Alcyonarians, the Antipatharians (= Gorgonacea) *Mopsea* and *Isis* as luminous. The exact identification of these forms is somewhat difficult, but Dr. Elizabeth Deichmann, who has looked up the original descriptions and comments on them by other writers, believes that "une magnifique Isis . . . pres d'un mètre de haut" taken off Santander in 564 meters was probably *Ceratoisis flexibilis* or *C. grayi* and that "des fragments de *Mopsea*" seen by de Folin were *Mopsea lofotensis*, now known as *Isidella elongata*. Whatever the identification, it seems certain that luminous Gorgonians exist. A *Mopsea* described by Moseley is now known as *Primiopsis*. On the other hand the luminous Alcyonium of Leuckart, mentioned by Mangold (1910), and the luminous Madre-

pora, mentioned by Gadeau de Kerville (1890, p. 50) are very dubious. Further study of these groups is much to be desired.

Pennatulacea

Luminescence of Zoophytes. Although the Romans knew of sea pens, referring to them as "*Penna marina*," the sea feather, or as "*Mentula alata*," the winged penis, the ability to luminesce does not appear to have been recorded. Probably the first mention of luminous sea pens comes to us in the famed "*De lunariis*" and the "*Historia Animalium*" of Conrad Gesner, published in the middle sixteenth century. Other early sixteenth and seventeenth century accounts are to be found in the writings of Rondelet, Boussuet, Aldrovandi, Bauhin, Boccone, and Imperati.

In the eighteenth century the light of the sea pen was recorded by Shaw in 1757 from the Barbary coast; by Bohadsch in 1761, who first described *Penna grisea* (*Pteroides griseum*); by Ellis in 1763, who wrote of *Pennatula phosphorea* and of the "kidney shaped purple sea pen from South Carolina," now called *Renilla*, by Pallas in 1766, by Spallanzani in 1783, and others. In the early nineteenth century luminescence of sea pens was mentioned by Tilesius (1819), Rapp (1828), Blainville (1834), and others.

The luminescence of zoophytes excited considerable interest among the British zoologists. Johnson (1847) published a letter from Edward Forbes, who drew the following conclusions from his study of *Pennatula phosphorea*: "1st. The polype is phosphorescent only when irritated by touch; 2d. The phosphorescence appears at the place touched, whether it be the stalk or the polypiferous part, and proceeds from thence in an undulating wave to the extremity of the polypiferous portion, and never in the other direction; 3d. If the centre of the polypiferous portion be touched, only those polypes above the touched part give out light; and if the extreme polypiferous pinna be touched, it alone of the whole animal exhibits the phenomenon of phosphorescence; 4th. The light is emitted for a longer time from the point of injury or pressure than from the other luminous parts; 5th. Sparks of light are sometimes sent out by the animals when pressed—these are found to arise from luminous matter investing ejected spicula."

Forbes also persuaded his friend George Wilson, the distinguished chemist, to determine if the luminescence was of electrical origin, and Wilson made a number of experiments to find out whether *Pennatula* evolved electricity like an influence machine or a voltaic battery. By insulating the *Pennatula* in the air or in turpentine and bringing it near a gold leaf electroscope or connecting it to a sensitive galvanometer, no indications of electric charge or current were obtained.

Wilson finally concluded: "On the whole I believe it most probable that the animal secretes a spontaneously inflammable substance. It may be a compound of phosphorous, but it is not necessary to assume that it is." The modern study of pennatulids begins with Panceri (1871) whose histological, biochemical, and physiological observations will be described shortly.

While all species of the Pennatulacea are not luminous, a very large proportion of them are. The best-known genera are *Pennatula*, *Pteroides*, *Veretillum*, *Renilla*, *Cavernularia*, and *Ptylosarcus*. Some of the deep water forms must present a wonderful sight. Herdman (1913) described a giant sea pen, *Funiculina quadrangularis*, 62 in. long, from 20 fathoms depth in the Firth of Lorn, with polyps nearly 1 in. long. "The long bare lower part of the stem, 9 inches to a foot in length, when gently stroked in the dark glows with a continuous sheet of light of (it seemed to me) a pale green colour which flickers or pulsates with a lambent flame." He also determined that *Virgularia mirabilis* from Loch Nevis was not luminous on stimulation.

Histology. Panceri (1871) first directed attention to the fact that the luminous slime coming from the surface of the polyps arose in special luminous structures, which he described as follows:¹² "The phosphorescent organs of the Pennatulæ consist of light cords (*cordoni luminosi*), which adhere to the external surface of the stomach of the polyps and the zooids, and are continued into each of the buccal papillae of both.

"These cords are principally composed of a substance contained in vesicles or cells, and which has all the characters of fatty matter, including that it does not decompose immediately after the putrefaction of the polyps. To these are added multipolar cells and albuminoid granulations.

"In the *Pennatula* phosphoria is found besides this a mineral substance, white, granular, and indefinite in its composition, but which is neither a carbonate nor a calcareous phosphate. This matter is wanting in the *Pennatula rubra*, in the *Pteroides griseum*, and in the *Funiculina quadrangularis*, which present, however, organs and luminous phenomena similar to those of the *P. phosphoria*; hence it results that no special importance can be attributed to them."

Bujar (1901) also, who studied *Veretillum*, figured granules of "fat" which contribute to the luminescence and small drops or "gouttelettes" which are analogous to the "vacuolides" of Dubois, supposed to contain the luciferase of Pholas. Niedermeyer (1911) in a long paper, dealing mainly with the anatomy and histology of *Pteroides*

¹² From the English translation in *Quart. J. Microscop. Sci.*, 12, 249, 1872.

griseum, could find no true light organ but flask-shaped gland cells from which the light presumably appeared. Like many other observers, including the author, Niedermeyer could not be certain whether the light was intra- or extracellular, but was inclined to think it intracellular because on normal stimulation he found no evidence of a secretion. When handled, a pennatulid does give off a luminous slime which sticks to the fingers, like the slime of medusae, but when

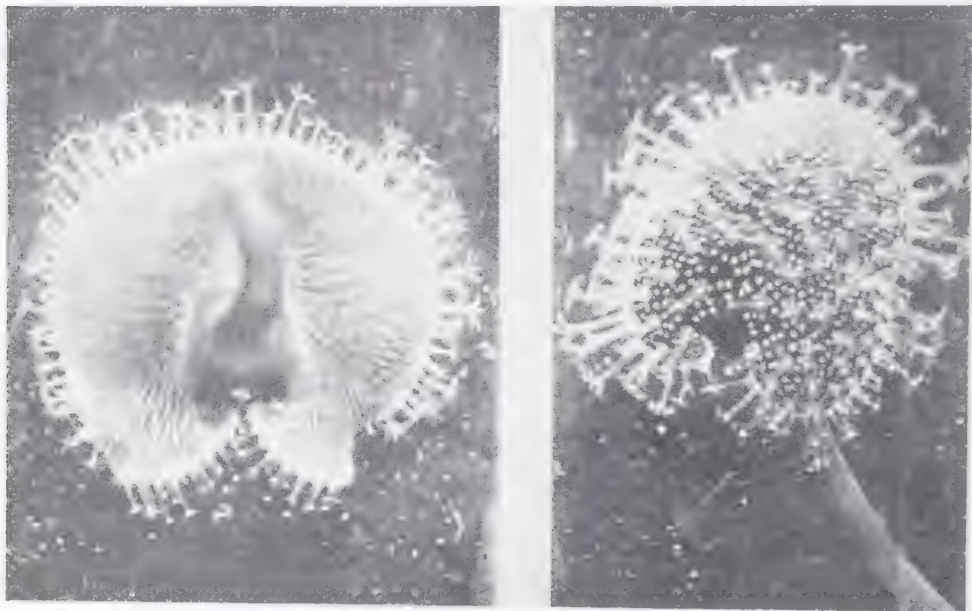


FIG. 48. The pennatulid, *Renilla amethystina*, showing individual zooids making up a kidney-shaped colony on a stalk. After Parker.

locally stimulated so that a wave of light passes over the colony, it is very difficult to note an external secretion.

In *Renilla amethystina* of the Pacific Coast, studied by Parker (1920) and shown in Fig. 48, the material associated with phosphorescence is of two kinds, a central whitish chalky material and a peripheral light yellow crystalline one. The two are usually rather intimately associated, but on the extreme edge of the rachis these materials form a double fringe, the outer fringe whitish and the inner composed of the yellowish cells. In this region it was possible for Parker to determine that light emission was associated with the whitish material. No histological work on *Renilla* has been carried out, and this gap in our knowledge should be filled.

The only histological study from sections is that of Hasama (1943) on *Cavernularia haberi*. Hasama emphasized the lack of a special luminous organ in this form and designed certain very small cells (much smaller than in the worm *Chaetopterus*), located between

numerous tufts (Zotten) which can be seen on the inner surface of the colony. These fine cells also occur on the inner surface of each polyp where there are spindle-shaped excretory structures with an inner lumen opening to the outside. However, Hasama's figures leave much to be desired.

Excitation and Transmission. The most striking aspect of pennatulid luminescence is a wave of light which passes over the colony when touched in a local spot. It was observed by Spallanzani, Blainville, Della Chiaje, and Forbes, but Panceri first made detailed observations of the possible direction and velocity of the waves. He pointed out that the colonies, living at depths of 40 to 100 meters, must be freshly collected for good results since, due to poor conditions in an aquarium, pennatulids absorb water and swell, sometimes to double their bulk.

"With colonies in good condition a stimulus at one point will result in what he called "luminous currents," "as if the little polyps took fire one after another, those on one branch before those of the following one. . . . If we operate on the basal extremity of the stalk, we shall have on the stem an ascending luminous current. If the stimulant is, on the contrary, applied to the top of the polypidom, there will be a descending current. Lastly, if the stimulus is applied to the feathered part of the rachis, one will then obtain two divergent currents. If the two extremities of the polypidom are simultaneously excited, one will have two currents convergent, which usually cease after a period of great vivacity at the point where they meet. I have only once seen in a very sensitive *Pennatula* the two converging currents continue after thus meeting, each one their own way, as if the other did not exist." Panceri's results thus differ from Forbes.

Panceri observed a latent period of $\frac{1}{5}$ second before "application of the excitement and the commencement of the (luminous) current." He then measured the velocity of propagation in both *Pennatula phosphora* and *P. rubra*, finding an average value of 5 cm per second (temperature not specified) which he took pains to point out is 600 times slower than the Helmholtz figure for the velocity of nerve conduction in the sciatic nerve of the frog. Another rather rare pennatulid, *Cavernularia pusilla*, behaved in the same way.

Panceri regarded the transmission of a luminous wave as most remarkable. He suspected that nerve fibers were involved but decided that further work would be necessary to determine whether the luminous current is actually transmitted along nerves. Since then the nerve net of coelenterates has become well known and all later writers have been equally intrigued by the propagation of the luminous wave.

The author (1917) has studied excitation in a pennatulid from Misaki, Japan, *Cavernularia haberi*, which sometimes attains a length of 2 ft when in the expanded condition. The living colony responds readily to electrical stimulation. There is usually no response to a single weak induction shock, but a local luminescence occurs after three or more shocks in rapid succession. With stronger stimuli, a wave of light, easily followed by the eye, passes over the colony in each direction from the point stimulated. With interrupted induced shocks, a series of waves of light follow one another in quick succession but do not correspond to the number of stimuli. The time interval between separate flashes no doubt represents the refractory period of the cells concerned.

On pressing deeply into the tissue and stimulating strongly, a much brighter light response results, which very slowly moves away from the point of stimulation but usually does not extend more than 2 or 3 cm. At the same time the whole colony contracts and the polyps are drawn in. In this contracted condition there is no response to electrical stimulation by light production. The slow wave of light above mentioned will pass in any direction over the colony and across a cut around the middle of the colony, involving the whole of the external tissue. Some inner tissue must therefore be capable of conducting this type of stimulus.

When a galvanic current is passed through one of the excised polyps mounted between non-polarizable electrodes, a flash of light occurs on the make and a series of flashes while the current is passing, with no flash of light on the break. A similar response can be observed with *Noctiluca* and is characteristic of other tissue, for example the series of contractions in the bell of a medusa during the passage of a galvanic current.

Somewhat similar behavior has been reported by Parker (1920) in an extensive study of the sea pansy, *Renilla*, in which two types of propagated wave occur. Spontaneous peristaltic waves of muscle contraction pass over the peduncle and also over the rachis, but no luminescence accompanies these waves. Electrical (or mechanical) stimulation of the rachis or peduncle at any point will produce luminous ripples which "spread concentrically over the rachis, like waves over the smooth surface of a pond into which a pebble has been thrown. If a fine needle point is used as a mechanical stimulus, a single point of light can be excited on the rachis, and this point will glow for some seconds and without becoming a center from which waves emanate, thus showing that in this instance the activity is strictly local."

The peristaltic and luminescence waves are quite distinct and can

pass each other without interference, evidently carried by different tissues. The rate is much faster for luminescent waves, 7.4 cm per second at 21° as compared with 0.2 mm per second for peristaltic waves. Between 10° and 25° the rate of luminous waves doubles for each increment of 10° , and at 31° is more than double that at 21° .

Although the luminescence is limited to regions containing the whitish material previously mentioned, Parker found that the impulses for luminescence are transmitted by the non-luminescing peduncle. These impulses will travel over any part of the intact surface of the colony. By making cuts in various directions, provided they do not isolate a region, the luminescence waves can be directed into a long and circuitous pathway.

In addition to the peristaltic waves and the luminescent waves there is a third system of wave propagation over the rachis which results in withdrawal of the autozooids. These impulses travel with a velocity of 7.8 per second. All three types of transmission can be temporarily interrupted by MgSO_4 .

Parker concluded that the peduncular and rachidial peristalsis transmission is probably muscular and the rhythm myogenic in origin, while the "phosphorescence, the withdrawal of autozooids and general contractions are called forth by impulses, often wave-like in character and probably neurogenic in origin (nerve net)." It is obvious that this colonial form offers unique material for a study of neuromuscular and neuroluminescent behavior. Honjo (1944), in a study of *Cavernularia obesa*, has obtained essentially the same results as Parker.

Inhibition. Panceri (1871) noticed that when two "luminous currents" met they were unable to pass, and the luminescence disappeared. Whenever impulses traveling in opposite directions in a nerve net, such as is characteristic of coelenterates, meet head on, they block and annihilate each other. This type of inhibition has been studied in *Penatula* by Moore (1926), who wrote as follows: "While the waves are running from base to tip as a result of gentle mechanical stimulation, if the tip of the colony then be stimulated by pressure of the finger, an area of darkness spreads from the tip to the base. Here are two seats of excitation but no luminescent waves are to be seen. Excitation at the tip has inhibited the effects of excitation at the base. If the exciting agent is withdrawn from one of the two loci then the waves of luminescence proceed from the remaining single point of stimulation as before. . . . The inhibition at times may be incomplete. Thus, if a single source of excitation yields luminescent waves at the rate of 3 per second, simultaneous stimulation at the other end of the animal reduces the number to 1 per second, i.e., the inhibition is partial."

Electroluminogram. In view of the complicated neuromuscular structure of pennatulids, the demonstration of electrical changes accompanying the appearance of luminescence is fraught with difficulties. Nevertheless, Hasama (1943) has published a record of such potential changes from bioluminescence, an electroluminogram of *Cavernularia habereri*. Hasama's figure shows a monophasic string galvanometer deflection when electrodes are placed on a luminescing and non luminescing region of the colony. The luminescent region is negative. The potential begins to appear about 1 second after mechanical stimulation and lasts about 5 seconds. No potential is found if leads are placed on two simultaneously luminescing spots.

Chemical stimulation of luminescence by alcohol, ether, or acetic acid also results in lasting potentials and lasting luminescence. Again the chemically stimulated luminous part is negative, an effect which might be due to injury potentials. Two brightly luminescing spots, chemically stimulated, are equipotential. Although Hasama realized that muscle contraction might produce potentials and spoke of mono- and diphasic potentials representing an electromyogram, superposed on the luminescence potential, these are not figured and their character is difficult to visualize. Hasama had previously observed potential changes accompanying luminescence of fire-flies, squid (*Watasenia*), worms (*Chaetopterus*), and nudibranchs (*Pleurophorus*).

Effect of Salts. The effect of varying the salt content of the medium on light emission of *Cavernularia habereri* has been studied by King-li-pin *et al.* (1935). The four principal salts of sea water were made up in distilled water in the following concentrations: 3% NaCl, 3.8% KCl, 6.8% $MgCl_2 \cdot 6H_2O$ and 7.5% $CaCl_2 \cdot 6H_2O$. Specimens of *Cavernularia* were then immersed in the pure salt solutions and in mixtures of equal volumes of the various salts and light emission was observed, usually after five minutes immersion. The results are given in the following table, where + means that luminescence appears and — means no luminescence.

NaCl	—	$CaCl_2 + MgCl_2$	—	$NaCl + KCl + MgCl_2$	+
KCl	+	$NaCl + MgCl_2$	—	$NaCl + KCl + CaCl_2$	+
$CaCl_2$	—	$KCl + CaCl_2$	+	$KCl + CaCl_2 + MgCl_2$	+
$MgCl_2$	—	$KCl + MgCl_2$	+	$NaCl + CaCl_2 + MgCl_2$	—
$NaCl + KCl$	+	$NaCl + CaCl_2$	—	$NaCl + KCl + CaCl_2 + MgCl_2$	+

It will be observed that whenever KCl is present the colony luminesces and that other cations do not excite the luminescence without K. In sea water the K is evidently present in too small a concentration to have an excitatory effect.

Effect of Illumination. Panceri (1871), stimulated by Allman's work on ctenophores, tested *Pennatula rubra* and *P. phosphorea* and found that their luminescence was not affected but that *Pteroides griseum* luminescence was inhibited by sunlight. The ability to luminesce again returned in the dark. The next investigator to study the effect of light on sea pens was Niedermeyer (1911), who found that at 9 A.M. and at noon specimens of *Pteroides* in a dark room would not luminesce on stimulation but later, after a stream of air had been passed through the sea water, a weak light appeared, and at 4 P.M. the luminescence was good. Niedermeyer did not mention the dark adaptation of his eyes, but Panceri's observations were apparently confirmed.

In view of the often contradictory reports on inhibition of luminescence by light in various species of luminous animals, the author (1926) has made a comparative study of available forms. It was found that at Naples, *Pennatula phosphorea*, brought from bright sunlight in December by an assistant to the dark room, would luminesce perfectly on stimulation. The same was true of another pennatulid, *Ptylosarcus* sp. dredged at Friday Harbor, Washington.

On the other hand Parker (1920) has reported that *Renilla amethystina* glows only at night, not in the daytime. Parker wrote: "During August in La Jolla this phosphorescence made its first appearance about half past eight o'clock in the evening and could be excited any time during the night until toward sunrise.

"If during daylight non-phosphorescent colonies are transferred to a dark room and kept there, they begin to show phosphorescence on stimulation in about half an hour and attain what seems to be their maximum capability under these circumstances in from fifty-five to sixty-five minutes. The phosphorescence thus developed seemed never to reach the degree of brightness seen during the night. . . . Phosphorescence induced during the daytime by placing a colony for an hour or so in the dark is completely lost on exposure to daylight for about five minutes. If during the night a colony that shows a naturally acquired bright phosphorescence is illuminated by a strong electric light (40-watt Mazda lamp at 40 cm distance), the ability to produce light steadily decreases. After five minutes' exposure to light the phosphorescence of the *Renilla* was obviously fainter than that of another kept in the dark as a check. And after ten minutes' exposure it was very faint in comparison. Continued exposure, however, never totally obliterated the light. . . .

The above experiments were repeated by F. B. Sumner in 1939.¹⁴

¹⁴ Private communication to G. H. Parker.

who found that freshly collected *Renillae* brought to a dark room in the morning, with the observer dark adapted, did luminesce when tested some two minutes after they had been placed in the dark but were not nearly as bright as ones which had remained in the dark room (in daytime) for an hour. On the other hand Summer observed that sometimes *Renillae* which have been in the aquarium for several months, although they look normal and contract when disturbed, gave only an occasional flash of light even when kept in the dark (in daytime) for over an hour. Judging from the results of these two observers, it seems certain that light does affect the luminescence of *Renilla*, although it may not inhibit it entirely, and that other factors, which require further study, are also involved.

Action of Drugs. Krukenberg's (1887) study of luminescence of plants and animals contains a section on *Pteroides griseum*, giving the results of stimulation to luminescence of quite a variety of chemicals, mostly alkaloids such as strychnine, veratrine, caffeine, and morphine. Unfortunately they were tested in only one concentration and are of little value. The scattered observations of Niedermeyer (1911) on the excitation of long-continued light by saturated HgCl_2 , 4% formalin, etc., do not add much to the analysis. As Coelenterates in general and pennatulids in particular are unusually favorable material for study of alkaloid action, it is surprising that more experiments have not been reported. Honjo (1944) has investigated strychnine effects, using *Cavernularia obesa*. In this form weak luminescence can be caused by stimulating any part of the colony but strong luminescence only by stimulating the peduncle. Strychnine in 1:100,000 concentration prevented the strong luminescence but not the weak; in 1:2,000 concentration, neither weak nor strong luminescence was possible. The living polyps can undoubtedly be narcotized, with reversible suppression of luminescence, but critical studies have not been made.

Biochemistry. In pennatulids, as in jelly-fish, *Beroë*, *Pholas*, *Chaetopterus*, and *Noctiluca*, Panceri observed a fundamental similarity in the behavior of the luminous material. In all these forms the luminous matter, after separation from the animal, may be induced to shine by the addition of fresh water. He continually referred to the luminous material as fatty, "a phosphoric grease." All subsequent observers have been impressed by the long-lasting ability of coelenterate material to emit light.

Such a luminous extract prepared from squeezed colonies of *Cavernularia haberi* by filtering through filter paper has been studied by Harvey (1917). After some time the luminescence of the fresh juice disappears but can be revived on addition of substances (saponin,

chloroform, benzol, oleic acid) many of which are cytolytic agents. Isotonic cane sugar solution does not revive the luminescence. There is no doubt that light production is intimately connected with the granules which the juice contains. After centrifuging, the heavy granule layer is found to be brilliantly phosphorescent on adding water, while the slightly turbid upper layer gives a much fainter light on dilution. No light can be obtained from the filtrate through a Pasteur-Chamberlain filter candle. The behavior is such as to suggest that solution of the granules (granulolysis) is accompanied by luminescence.

Attempts to obtain luminous materials from the juice were not very successful. If to the fresh filtered luminous juice of *Cavernularia* there is added sugar, NaCl , MgSO_4 , $(\text{NH}_4)_2\text{SO}_4$ to saturation, or 5 volumes of glycerine, the light disappears. A heavy precipitate forms in $(\text{NH}_4)_2\text{SO}_4$, a small precipitate in MgSO_4 , and practically none in NaCl , and none in sugar or glycerine. The precipitates are soluble in sea water and if poured into fresh water or sea water immediately after the light has disappeared, light is produced by all solutions. If the tubes are allowed to stand for a day, no light is produced by any tube on pouring into fresh water or sea water.

When alcohol or acetone (3 volumes) or alkaloidal reagents are added to the juice, a precipitate forms, and the light disappears. The precipitate is insoluble and gives no light in sea water or fresh water.

Attempts to extinguish reversibly the luminescence of the *Cavernularia* juice by such narcotics as ether, chloroform, ethyl or butyl alcohol have failed. Concentrations which cause disappearance of the light produce irreversible effects, for no return of light appears on removing the narcotic or diluting the narcotic-juice mixture. In this respect the luminous juice differs from many luminous cells (*Noctiluca* or luminous bacteria) which can be truly narcotized.

No luciferin-luciferase reaction could be demonstrated with *Cavernularia* extracts (Harvey, 1916) nor with other pennatulids that have been tested, such as *Ptylosarcus* sp. (Harvey, 1921) or *Pennatula phosphorea* (Harvey, 1926) even when the luciferin solutions were prepared in absence of oxygen. Neither would extracts of these forms, so prepared that they should contain luciferin (or luciferase), give luminescence when mixed with *Cypridina* luciferase (or luciferin).

Luminous *Cavernularia* juice must have a considerable oxygen consumption, for a test tube filled with the luminescent juice soon becomes dark, except at the top in contact with air. Shaking the tube with air will restore the luminescence. Moreover, on passing hydrogen through a vessel containing *Cavernularia* juice, the luminescence quickly dis-

appears, to return on admitting oxygen. Similar experiments have indicated that *Pennatula phosphorea* also requires oxygen for luminescence. In this respect pennatulids differ from *Radiolaria*, *Pelagia*, and the ctenophores, whose light still continues in complete absence of oxygen.

As is the case with so many luminous forms cyanide has no special inhibitory action on luminescence comparable to its effect in suppressing oxygen consumption of many cells. KCN, added in as high as 0.02 *m* concentration, does not suppress the light of *Cavernularia* juice even after twenty minutes.

Examined in ultraviolet light (without the visible) neither *Pennatula phosphorea* nor *Funiculina quadrangularis* exhibit any special fluorescence of luminous material, although the skeleton of both forms is highly fluorescent (Harvey, 1926). The fluorescence of *Renilla*, where two materials closely associated with luminescence are easily visible, should be studied with the fluorescence microscope.

Physical Characteristics. The only data on the special quality of Anthozoan luminescence is quite old, for example the observations of Panceri (1871), who studied a number of marine forms including *Pennatula polyps* immersed in fresh water. His spectroscope was not of sufficient aperture to show more than a faint bluish or greenish band with no color or lines. Moseley (1877) examined three species of Alcyonarians, all phosphorescent, and found only red, yellow, and green rays in the spectrum. Blue and violet were absent and Moseley pointed out that no blue-colored deep sea animals were caught on the *Challenger* expedition, although shallow water blue animals are not uncommon. No spectral energy curve of any of the coelenterates has been published, a gap in our knowledge that could be easily filled with modern spectroscopic techniques.

CHAPTER V

Ctenophora

INTRODUCTION

Among other characteristics the ctenophores or comb-bearers differ from the Cnidaria by the presence of fused cilia which form the swimming plates or combs and by absence of nettle cells. A common type found along the Atlantic coast is shown in Fig. 49. All ctenophores are marine and transparent, without hard skeletal structures and all may be luminous, at least all those which have been properly tested will luminesce. The phylum is a small one, with less than one hundred species.

The first discovery of luminescence in a ctenophore is difficult to place. There appear to be no certain references in Roman times but they were often confused with and spoken of as medusae. According to Chun (1880), Friedrich Martens, a ship's surgeon, saw ctenophores in the neighborhood of Spitzbergen in 1671, and Patrick Brown near Jamaica in 1756, but neither mentioned luminescence. Linné included two species in the tenth edition of *Systema naturae*, *Volvox beroë* and *Volvox bicaudatus*. Again there is no mention of luminescence. The discovery of luminescence is undoubtedly connected with early voyageurs and the general study of sea phosphorescence. It is certain that ctenophores were observed to be luminous by Bosc (1800), Mitchill (1801),¹ Tilesius (1815), Rang (1829), Della Chiaje (1834), Will (1844), Forbes (1848), Milne-Edwards (1863), Giglioli (1870), and many others.

The classification of T. Krumbach follows. Only the genera containing known luminous species are italicized, but it is probable that many more are luminous.

Ctenophora

Cydroppea

Haeckeliidae (*Haeckelia* = *Euchlora rubra*)

Euchoridae (*Euchlora filigera*, *Dryodora*)

¹ The animal Mitchill called *Medusa simplex* was undoubtedly a ctenophore.

Mertensiidae (Mertensia)

Callianiridae (Callianira)

Pleurobranchiidae (Pleurobranchia, Hormiphora)

Eulampetiidae (Eulampetia, Euplokamis)

Bathyctenidae (Bathyctena, Aulacoctena) deep sea



FIG. 49. The ctenophore, *Mnemiopsis leidyi* by day. The regularly spaced swimming plates (on eight radial canals) are clearly shown. Photo by G. Lowe.

Tjalfiellidea

Tjalfiellidae (Tjalfiella) deep sea

Cestidea

Cestidae (*Cestum*, Velamen = Vexillum = Folia)

Ganeshidae (Ganesha)

Bolinopsidea

Bolinopsidae (*Bolinopsis*? = *Bolina*)

Ocyropsidae (*Ocyropsis* = *Ocyroë* = *Calymma*)

Mutilated individuals of *Bolinopsis* have been described as *Lesueurina*. Old descriptions of *Alcinoë* probably referred to *Mnemiopsis* or *Leucothea*; *Chiaja* and *Eschscholtzia* to *Leucothea*; *Cydippe* to *Hormiphora*; *Mnemia* to *Mnemiopsis*.

Mnemiopsidae (*Mnemiopsis*)

?*Deiopeidae* (?*Deiopea*)

Eurhamphaeidae (*Eurhamphaea*)

Leucotheidae or *Eucharidae* (*Leucothea* = *Eucharis*)

Ctenoplanidea

Ctenoplanidae (*Ctenoplana*)

Coeloplanidae (*Coeloplana*)

Beroidea

Beroidae (*Beroë*, *Pandora*, *Neis*)

As with so many other marine forms, the modern work may be said to begin with Panceri (1872) whose extensive paper mentioned a number of luminous species: *Beroë albens*, *B. rufescens*, *Cydlippe densa*, *Bolina hibernica*, *Alcynoë papillosa*, *Eschscholtia cordata*, and *Cestus veneris*. Panceri dealt with the position of luminous organs, the effect of various stimuli, physical and chemical, especially fresh water in exciting the luminescence, and the effect of light in inhibiting the luminescence. He also noted the color of the light and attempted to determine its spectrum. In short Panceri laid the morphological and physiological groundwork for understanding the luminescence of these forms.

The great monograph of Chun (1884) was largely morphological and systematic, but two pages were devoted to "Das Leuchten der Rippenquallen," which included his own opinion on luminescence. The later physiological and biochemical work has been largely carried out by Peters (1905), Harvey (1921, 25), Moore (1923, 24, 26), Heymans and Moore (1925), Harvey and Korr (1938), and Chase (1941).

LUMINESCENT EGGS

It was recognized by Allman (1862) that the developing eggs and embryos of *Beroë* were luminous. He thought that a considerable amount of sea phosphorescence might come from this source. Alexander Agassiz (1874, p. 371) a little later confirmed the observation. He wrote: "We know now something of the nature of phosphorescence of a few marine Invertebrates from the observations of Panceri, who has plainly traced it to the secretion of special glands, yet when we find the same phosphorescence equally as brilliant in eggs of Ctenophorae as in adults, even in stages in which the masses of segmentation can still be counted, we have evidently not yet reached the solution of the true nature of this phosphorescence. The whole embryonic mass becomes brilliantly phosphorescent when the least shock is given to the jar in which the eggs are kept."

Peters (1905), who noticed that the shedding of eggs was retarded by light, also determined that the early segmentation stages of *Mnemiopsis leidyi*, before the cilia appeared, were luminous, but the

unsegmented egg was non luminous. In other forms the unsegmented eggs are luminous. Yatsu (1912) has reported that the egg of *Beroë* at Naples is covered by a thick layer of finely alveolar ectoplasm which



FIG. 50. Two specimens of *Mnemiopsis* photographed by a flash-bulb (above, and by their own luminescence after electrical stimulation (below). Only four luminous canals show clearly. The screen electrodes for stimulation are visible at the sides of the photos, taken by G. Lower and the author.

"is fluorescent and looks green under reflected light reminding one of a piece of uranium glass." When stimulated "with a weak electric current this layer alone seems to emit a beautiful greenish light," confined to the ectoplasmic layer as development proceeds. Okada (1926) could

find no granules, such as are usually associated with luminescence, in the ectoplasm of *Ocyropsis fusca* but stated that light is emitted by this egg.

MORPHOLOGY

In many adult ctenophores it is easy to observe that luminescence is confined to the eight radial canals, which are covered externally by the swimming plates, as illustrated in Fig. 50. Peters (1905) found that in *Mnemiopsis* luminescence could be obtained from the tissue

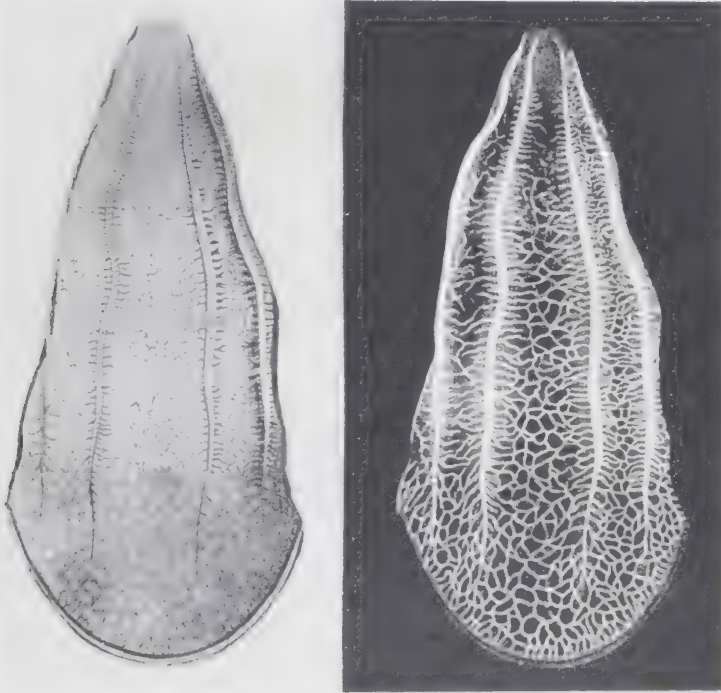


FIG. 51. *Beroë* by daylight and at night showing four of the eight meridional canals and the network of interconnecting canals. After Panceri.

containing as few as four isolated paddle plates but not from fewer than four and not from the aboral sense organ or the auricles covered with separate rows of cilia. The author (1925) has observed that less than two paddle plates will luminesce, and the connection of luminescence with paddle plates is purely coincidental. For example, in *Beroë rufescens*, the network of small interconnecting canals away from swimming plates is highly luminescent, as shown in Fig. 51. Okada (1926) also noted that the luminescence of *Ocyropsis fusca*, found in the spring at Misaki, Japan, is especially bright in the subpharyngeal

¹The modern name is *Beroë forskala*. Another species, called by Panceri, *Beroë albens*, now known as *B. ovata*, lights only along meridional canals.

meridional canals of the well-developed lappets of this form. These canals are far removed from any swimming plates. The luminescence came from the canals, not the adjacent tissue, and was "strictly limited to the region where the sexual cells are found."

In *Cestus veneris* the body is enormously flattened in the plane of the tentacles so that it appears like a ribbon sometimes a meter long. Panceri has described the canals of the two superior ribs as well as the inferior marginal canal as luminous.

According to Dawydoff (1946), *Hormiphora luminosa* from Indo-china seas shows two remarkable organs which produce light. Observed in the dark, "Les Hormiphores en question montrent toujours deux points phosphorescents tres nets," but Dawydoff gave no further details and the histology was not studied.

HISTOLOGY

Panceri (1872) described the luminous parts of the canals as containing a yellowish fatty globular material. Chun (1884) has pointed out, however, that the vessels of ctenophores never contain cells, and he has identified the luminous region as made up of "strongly vacuolated endodermal cells filled with fat droplets, which are developed as enlargements of the peripheral vessels. Inasmuch as the sex organs are only a modified part of the thickened vessel wall, I would not be surprised to find that the intensive luminescence is to be attributed to the ovarial and spermatic bands. In sexually mature *Beroë ovata* the luminescence appears in double luminous stripes corresponding to the position of the sex products." Chun then proceeded to cite the observations of Allman (1862) and Agassiz (1874) on the luminescence of ctenophore eggs as evidence for his idea that light was connected with the sex cells. The light cells are certainly within the canals, for no luminescent slime sticks to the fingers if the outer surface of a ctenophore is gently stroked.

Dahlgren (1916) has studied *Pleurobrachia pileus*, making sections of the canals which are reproduced as Fig. 52. He believed that the luminous cells formed a layer covering the sex cells, but distinct from them. Under high magnification the photogenic cells were highly vacuolated with scattered small granules near the free edge and a peculiar irregular dark staining body next to the granules. Although the cells were of a glandular type, Dahlgren believed the luminescence to be intra-cellular.

Much more work is needed to identify the luminous components. Since the ability of ctenophores to luminesce is abolished in daylight, it would appear that a comparison of the histology of animal tissue

fixed in daylight with that fixed in darkness should give some valuable information.

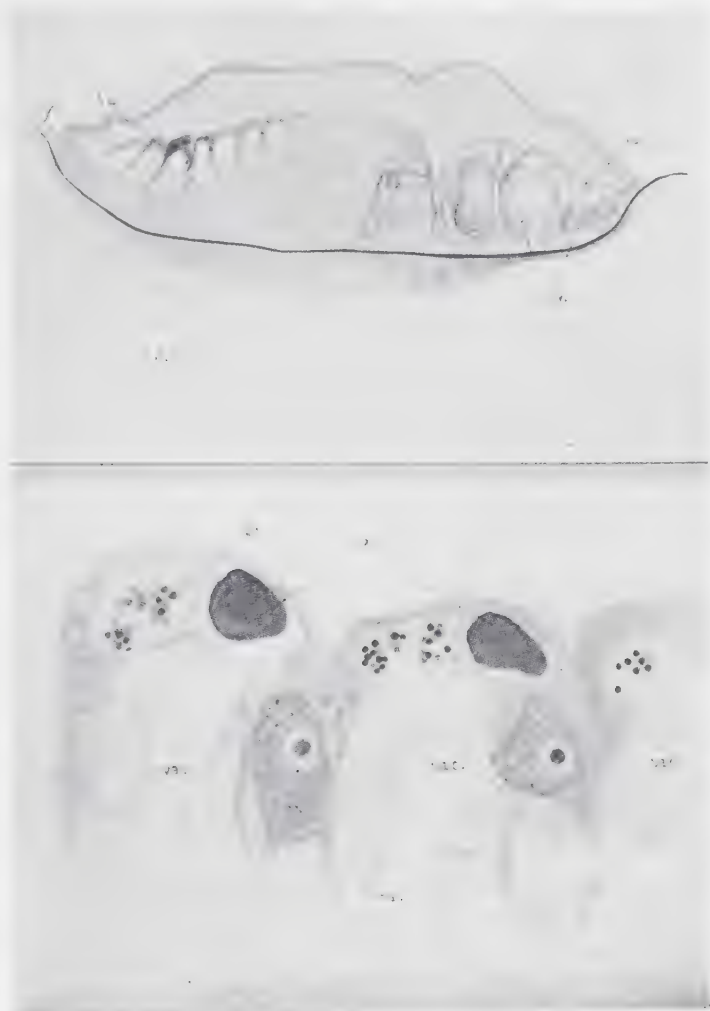


FIG. 52. Transverse section of one of the canals of *Pleurobrachia pileus* showing ovary (ov) and luminous cells, lu, (above) and an enlarged view of two luminous cells (below). x is an unknown structure always found near granules, gr; vac, the vacuole; nu, nucleus. After Dahlgren.

PHYSIOLOGY

Motor reactions in ctenophores involve the swimming plates and muscles. Rhythmic waves of excitation proceed from the aboral "sense organ" and pass orally along the eight meridional rows of paddle plates, resulting in the easily observed movement of one plate after another. Muscular contraction in *Mnemiopsis* is largely confined to closure of the oral lobes. It can be initiated by a touch anywhere on the animal.

which also involves cessation of movement of the swimming plates. Evidently the animal possesses a nerve muscle net system like that in a medusa.

Light emission is never spontaneous but only on stimulation of the luminescent cells in the eight meridional canals, a more restricted reflex. Moore (1924) has shown that "receptors for mechanical stimulation of the luminescent organs are limited in their distribution to the eight rows of paddle plates, because, while a touch with a glass needle applied to a meridian causes an immediate glow along that particular row of paddle plates, similar stimulation applied to the intermeridian does not result in luminescence unless the pressure applied is sufficient to cause some deformation of the meridian." Conducting tissue for luminescence extends along the eight meridians and waves of luminescence can move in either direction, but will not pass a cut dividing the meridional canal. Ctenophores offer superb material for study of nerve muscle and nerve-photogenic cell physiology. In addition to the mechanical receptors and chemical receptors, there are also light receptors which cause inhibition of luminescence. They involve a different set of nerve fibers and will be more fully discussed in the section "Effects of Illumination."

Electrical excitation of luminescence is also effective, and its analysis is fairly complicated. Moore (1926) has studied electric stimulation by a constant current. When a whole *Beroë* or *Mnemiopsis* was placed in a rectangular vessel and a galvanic current of a few milliamperes was passed through, Moore observed a luminescent glow on the anode side of the vessel on the make of the current. The glow lasted for some seconds during the current flow. In *Mnemiopsis*, a break flash could sometimes be observed at the cathode. When an incision was made in the animal, transversely with reference to the direction of the current, anodal stimulation occurred at the cut surface also. Moore wrote: "These results mean that galvanic stimulation takes place only at the protoplasm-sea water surface, and that stimulation is referable to the blocking of positive ions of the sea water at that surface. These ions, therefore, impinge on the protoplasmic membrane from outside. It is a remarkable fact that in ctenophores which consist so largely of included sea water there is no apparent stimulation as a result of internal ionic movements. This conclusion is necessary since stimulation appears only along the surface of the animal next the anode." The reversed law of Pflüger thus holds for ctenophores and is essentially the same as found by Moore (1926) for *Pelagia noctiluca*.

Stimulation of ctenophores to flash is abolished by lack of oxygen, and in this respect differs from luminescence of the photogenic material

itself, which can take place in complete absence of oxygen. A study of these effects has been made by Chase (1941), whose experiments concern the intact living animals. Chase wrote: "After complete de-aeration (thirty to forty minutes flushing with purified hydrogen) no flashing can be elicited by electrical or mechanical stimulation. Three to five minutes after re-admitting air the animal again responds. The cycle of de-aeration and re-aeration can be repeated as many as three times before the animal dies. Movement of the swimming plates stops at about the same time that luminescence on electrical stimulation ceases. As the animal begins to disintegrate, either in an atmosphere of air or of hydrogen, a dim, continuous luminescence gradually appears along rows of swimming plates. This lasts for about an hour, and undoubtedly represents the basic luminescent reaction as studied by Harvey and Korr, freed from its normal nervous control."

Chase (1941) also studied the effect of KCN and a few drugs on excitation of luminescence in the whole animal. He wrote: "Mnemiopsis in sea water (air present), loses its ability to luminesce on electrical stimulation within 15 seconds after addition of 0.0001 *M* KCN, although the swimming plates continue to move for ten to twenty minutes. Eserine (1:2,000) increases the sensitivity to luminesce on mechanical stimulation and also increases the duration of the luminescent flashes. Addition of acetylcholine (1:3,000) enhances this effect. Returning the animals to plain sea water gradually restores the normal response. Adrenaline (1:100,000) apparently decreases the sensitivity, but the effect is less clear-cut than the increased sensitivity caused by eserine and acetylcholine."

EFFECT OF ILLUMINATION

Another important discovery of Allman (1862), in addition to the existence of luminous segmentation stages in ctenophores, was the effect of light on the luminescence. No luminescence could be obtained on stimulating specimens of *Beroë* which had been exposed to daylight until they had been in the dark for some twenty minutes. Segmentation stages also would not luminesce if exposed to daylight. Allman stated that he was well aware that the eye must be dark adapted for the observations and took precautions to make sure that his own eyes were dark adapted. All observers since Allman, Panceri (1872), Peters (1905), Moore (1923-25), Heymans and Moore (1925), Harvey (1921, 25), and others, have observed the inhibition of ctenophore luminescence in the light and its return in the dark.

Ctenophores that have been in the light for some time will be called "light adapted" ctenophores. Peters (1905) observed that mechanical

stimulation accelerated the return of luminescence in light adapted ctenophores when placed in darkness although light adapted ctenophores did not become luminous by mechanical agitation alone. He was also aware that mechanical stimulation reduced the intensity of luminescence but did not easily inhibit it entirely.

A rather fragile and easily fatigued ctenophore, *Bolina* sp., which occurs at Friday Harbor, Washington, was investigated by the author (1921). It was found to be sensitive to light, losing its ability to luminesce, but regaining the power after thirty minutes in the dark. The question arose as to whether the effect of light acted on the stimulating mechanism for luminescence or actually caused disappearance of photogenic material. This question was tested by squeezing light-adapted *Bolinas* through four layers of cheesecloth to break up nerve connections thoroughly. No light appeared during the squeezing and no light appeared on adding the resultant extract of the *Bolinas* to fresh water. Hence no photogenic material was present. Such an extract from dark-adapted *Bolinas*, when added to fresh water, will luminesce brilliantly. It would therefore appear that the effect of light is actually to decompose photogenic substances in the cells. Although light-adapted whole *Bolinas* regain their ability to luminesce after one half hour in the dark, the extract of light-adapted *Bolinas*, kept in the dark for one half hour, does not luminesce when added to fresh water, i.e., recovery does not take place in the extract.

However, the author (1925) has observed a partial recovery of similar extracts of light-adapted *Mnemiopsis leidyi*, a much less fragile and more brilliantly luminescent ctenophore, after standing in the dark for some time. Extracts of dark-adapted *Mnemiopsis*, which have been exposed to light, emit no luminescence¹ with fresh water but regain that ability if placed in the dark. The extracts of this form, containing isolated cells or photogenic granules, are highly light sensitive, just as is the whole animal.

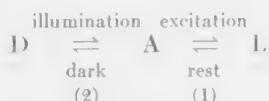
In addition to the direct action of light on photogenic material there is another effect of light on the luminescence excitatory mechanism. Moore (1924) has discovered that light, not strong enough to prevent luminescence of the luminous material itself, can affect the nervous control of luminescence in *Mnemiopsis*. Suppression of luminescence by weak light in the whole animal must involve photoreceptor cells and nerve connections. The relation between time and intensity for suppression of light by the whole animal obeys the Bunsen-Roscoe law, re-

¹ Sometimes a faint luminescence can be observed when light adapted ctenophores are squeezed through cheesecloth or when the extract is poured into fresh water. The inhibition is not absolutely complete.

quiring an exposure of some 4.776 meter-candle minutes. This effect of light is local, only the illuminated region having its luminescence suppressed. Another ctenophore, a *Beroë*, obtained by Heymans and Moore (1925) at Barnegat Bay, New Jersey, behaved as did *Mnemiopsis*, but required an exposure of 57.285 meter-candle minutes to inhibit luminescence of the intact animal, while *Cestus veneris* required 1.167 meter-candle minutes (Moore, 1926).

Moore (1925, 26) has also studied the rate at which the luminescent material of *Eucharis multicornis*, common at Naples, disappears on exposure to light. For this purpose the *Eucharis* was broken up by shaking in a flask, and the material was filtered through filter paper. The extract was then exposed to light and from time to time samples were removed to a dark room and added to distilled water in a vessel covered by a photographic plate. Later the plates were all developed simultaneously, and the blackening was used as a measure of the luminescence intensity of the *Mnemiopsis* extract after a given time of light exposure. It was found that the photolytic reaction conformed to the Bunsen-Roscoe law, that it proceeded as a first order reaction, and that rise in temperature between 7° and 22° did not materially change the rate, thus agreeing with the small effect of temperature found for many photochemical reactions.

Moore (1924) has visualized the luminescent system in ctenophores as involving the following reactions:



in which A is the luminescent substance in the resting, dark adapted animal, L is the light-giving form of the substance, formation of which is catalyzed by stimulation, D is a decomposition product, formation of which is facilitated by illumination of sufficient intensity and duration. Such a scheme makes intelligible the experiment of Peters in which he found that stimulation hastens the recovery of luminescence in an animal which had previously been light adapted, since excitation removes A from the system as rapidly as it is formed and thus facilitates the 'dark' reaction. Likewise the 'rest' reaction goes on even under strong illumination. It is evident that D cannot be directly converted into L, and it also seems probable that L cannot be changed into D without passing through the intermediary stage A.

If we assume that both reactions involving the decomposition of A are set up by nervous impulses, then each luminescent organ must receive two types of innervation. A nerve impulse arriving from a tactile

receptor sets going reaction (1) and luminescence results, but an impulse coming from the light receptor causes A to decompose reaction (2) into an altogether different substance, D, which cannot give rise to luminescence until it has been reformed into substance A. The luminescent substance thus receives double innervation and the character of the decomposition is determined by the type of nerve fibre stimulated."

Practically no work has been carried out to determine the wavelength of the luminescence-inhibiting light. Its action spectrum is unknown. The author (1925) made some preliminary tests which indicated that the violet end of the spectrum was more effective than the red end and definitely determined that near ultraviolet light (without the visible, mostly at 3650 Å) will inhibit the luminescence of ctenophores. The purpose of the experiment was to find out if luminescence-inhibiting radiation acted by accelerating the luminescent oxidation of the photogenic material. A bioluminescence that could not be observed when a ctenophore was exposed to sunlight or electric light would be easily visible when ultraviolet alone was used. It was found that the *Mnemiopsis* never showed any bioluminescence while exposed to the near ultraviolet light for a period of thirty minutes, after which the ctenophore had completely lost its ability to luminesce on stimulation.

Ultraviolet light does not stimulate to luminescence, but a very interesting phenomenon appears when the animal is mechanically stimulated while exposed to the ultraviolet. If stimulated several times, a decidedly bluish luminescence *persists* after the bioluminescence due to stimulation has subsided. This "tonic luminescence," disappears when the ultraviolet light is screened and reappears when the ultraviolet again strikes the animal and is evidently a fluorescence. If the animal has been previously stimulated in the dark and then placed in ultraviolet light, the "tonic luminescence" is observed and lasts for some time but gradually fades out.

There are two possible explanations of the "tonic luminescence." (1) It is a true chemiluminescence due to continuous or tonic stimulation of the photogenic cells by the ultraviolet light, after their luminescence has been started by mechanical stimulation. (2) The product of oxidation of photogenic material is fluorescent.

Certainly the behavior of this "tonic luminescence" is like a fluorescence, appearing only in the exciting radiation, but at the same time it is always associated with potentially luminescent animals. Light-adapted animals, previously exposed to sunlight, do not show "tonic luminescence" when stimulated before the ultraviolet treatment. Therefore, the effect is not due to fluorescence of any non-luminescent

mucus, secreted along the paddle plates on stimulation. Observation with a microscope shows that the "tonic luminescence" is in the canal, in the same position as the true luminescence of the animal.

Animals partially fatigued by gentle shaking for two minutes in a bottle exhibit "tonic luminescence" in the ultraviolet. If shaken in a bottle for fifteen minutes, when luminescence on stimulation is no longer possible, the "tonic luminescence" in ultraviolet is no longer apparent. Presumably some material must have diffused out into the sea water when agitated for a long time. The above experiments leave little doubt but that the so called "tonic luminescence" is a fluorescence of an oxidation product or some intermediate substance formed during the luminescence of the animal. The close relation between fluorescence and chemiluminescence warrants further investigation and isolation of this material.

RELATION TO OXYGEN

The most unusual chemical characteristic of ctenophore luminescence is its independence of dissolved oxygen. The author first observed this behavior, also shared by *Pelagia* and *Radiolaria*, in a systematic study (1926) of oxygen and luminescence in various luminous animals found at Naples. Since then, the experiments have been repeated many times, and a particularly careful study was made on *Mnemiopsis* by Harvey and Korr (1938), with the same result—*Mnemiopsis* extracts will luminesce with no more oxygen present than can exist at room temperature in the presence of hydrogen and platinized asbestos or in sodium hydrosulfite solutions. Under both conditions, safranin remains completely reduced.

Confirmation was also obtained of an older observation (Harvey, 1926) that the inhibitory action of light on ctenophore extracts takes place in complete absence of oxygen, and the additional important discovery was made that the ability to luminesce will not return in light-adapted extracts in absence of oxygen, although recovery does occur in presence of oxygen. Such behavior suggests that perhaps oxygen must combine with a precursor to form a photogen-oxygen compound which emits light when the photogenic granules of the ctenophore dissolve. This explanation also implies that the oxygen is bound so firmly to the photogen that it cannot be removed by sodium hydrosulfite or by hydrogen in presence of finely divided platinum.

BIOCHEMISTRY

Although Panceri (1872) described the luminous material of *Beroë* as a yellowish fatty substance, the author was unable to detect any yellow cells in *Bolina* or *Mnemiopsis* similar to the yellow masses in

the luminescent spots of the medusae, *Aequorea*, and *Halistaura*. Actually, nothing is known of the luminous material of ctenophores except that there is a considerable amount present and that these animals, like medusae and pennatulids, are favorable for biochemical work.

Ctenophore extracts behave in much the same way as extracts of medusae or pennatulids, previously described. The author (1921) has emphasized the brilliant luminescence which appears when the osmotic pressure of the extract is decreased or when cytolytic agents are added, while Moore (1924, 25), using both *Beroë* and *Mnemiopsis*, has found the same behavior of ctenophore luminous material on filter paper in pure salt solutions as in the case of similar "indicator paper" containing slime of *Pelagia noctiluca*. The *Mnemiopsis* indicator paper glowed in isotonic solutions of K_2SO_4 , KCl, CaCl₂, SrCl₂, and $MgSO_4$, brightest in the order mentioned, but not in NaCl and MgCl₂ or sea water itself. Variation in pH between 6 and 8 did not affect the result.

It has not been possible to demonstrate a luciferin-luciferase reaction with extracts of various species of *Beroë*, *Eucharis*, *Bolina*, or *Mnemiopsis*, even when the luciferin solution was prepared in absence of oxygen (Harvey, 1926, 31). Neither do extracts of ctenophores, so prepared that they should contain luciferin (or luciferase), give light when mixed with *Cypridina* luciferase (or luciferin).

In view of the importance of adenosine triphosphate (ATP) in fire-fly luminescence, the author (1949) tested this material with *Mnemiopsis* extract. Addition of ATP to an extract of fire-fly lanterns whose luminescence has disappeared will immediately revive the light, but no such revival of light occurs when ATP in sea water is added to an extract of *Mnemiopsis*. However, the *Mnemiopsis* extract contains abundant potential luminosity, as a brilliant light emission occurs when it is poured into fresh water.

CHAPTER VI

Miscellaneous Small Groups

TURBELLARIA

The great phylum of Platyhelminthes is divided into the flat-worms proper or Turbellaria, the parasitic flukes or Trematodes, and the tape-worms or Cestodes, also parasitic. Only the first group contains alleged luminous forms. The Turbellaria are unsegmented, living mostly in fresh or salt water, a few on the land. Some 1,500 species are known, grouped in 6 orders and 81 families.

The oldest record of a luminous flat-worm is that of Viviani (1805), described in his essay, "Phosphorescentia maris." He called the animal, *Planaria retusa*, and published a figure which could represent a flat-worm, although it might also be taken for a nudibranch. Viviani's *Planaria* was found on the branches of algae in the gulf of Genoa. Gadeau de Kerville (1890, p. 71) was inclined to consider the observation questionable and suggested that the light might be due to infection with luminous bacteria.

The second reference is by Noll (1879) who ascribed some of the bluish luminescence observed in his sea water aquarium to *Strudelwürmer* or Turbellaria. This observation is definitely questionable.

The third report comes from a book on marine animals, "Between Pacific Tides," by Ricketts and Calvin (1939, revised edition 1948). These authors state that in tide pools with red algae near Pacific Grove, California, a rhabdocoele flat-worm, *Monocelis*, can be seen as "little dots of light . . . crawling rapidly along the under side of the air-water film or swimming about the surface. Certain small annelid worms, likewise luminescent, may be found also under similar circumstances, but they swim about more rapidly, change direction quickly in darting movements, and are generally larger." This observation is perfectly definite, but confirmation is needed and particularly a detailed study of the luminous organ. *Monocelis* is one of 12 genera in the Monocelididae, of the order Alloeocoela, containing 11 families.

NEMERTINEA OR RHYNCHOCOELA

Introduction

There is no doubt of the existence of luminous nemerteans, although the group is, perhaps, one of the most unlikely phyla to have developed the ability to produce light. The Nemertinea, like the Platyhelminthes, are unsegmented and have no body cavity, the space between the viscera being filled with spongy tissue. They are exclusively marine, usually very thin and thread-like, and frequently attain enormous lengths.



FIG. 53. The nemertean, *Emplectonema kandai*. After Kato.

Some 500 species are known, grouped in four orders, according to the classification of L. Böhmig. The only known luminous genus is italicized.

Anopla

Palaeonemertini (5 families)

Heteronemertini (3 families)

Enopla

Hoploneimertini (17 families including Emplectonematidae or Nemertidae with (*Emplectonema*, *Nemertopsis*, *Paranemertes*, *Carcinonemertes*, *Gononemertes*)

Bdellonemertini

Malacobdellidae (1 genus)

The discovery of nemertean luminescence is quite recent, made by Kanda (1939) in 1936 on a visit to the Asamushi Biological Station on Aomori Bay, Japan. The animals, named *Emplectonema Kandai* by Kato (1939), were found living at 30 to 40 meters, coiled up in a common ascidian, *Chelyosoma siboja*, on the sandy or muddy bottom. Only 6 or 7 individuals at best, sometimes only one or two among 200 ascidians, collected by the fishermen as the result of a day's work, would contain *Emplectonema*, shown in Fig. 53.

The animals are 53 to 115 cm long and only 0.5 to 0.7 mm in diameter, of a reddish orange color. In captivity, they coil up on the wall or bottom of an aquarium, attached by the slime which is abun-

dantly secreted from the surface of the body. Kanda reported, "The animals flash brilliantly only on stimulation. The stimulus may be mechanical, chemical, thermal or electrical. The light may appear on all parts of the body [except the tip of the head], but it disappears in one or two seconds. It is whitish green in color."

The genus *Emplectonema* of the Hoplonemertini is widely distributed in America, both Atlantic and Pacific Oceans, the Mediterranean Sea, the White Sea, and around the coasts of Japan. Other luminous nemerteans must exist, as it would be surprising if this species was the only luminous one among fifteen of the genus that have been reported from various parts of the world and indeed the only one among the some 500 species of known nemerteans. The position of *Emplectonema* is shown in the classification of L. Böhmig.

Histology

The anatomy and some histology of *Emplectonema* has been described by Kato (1939), who merely mentioned that at least three kinds of gland cells which stain differently are present in the epidermis. However, Kanda (1939) has published photographs of sections, prepared by Atoda and Kato, which show cells staining with eosin and others with Delafield's hematoxylin. Kanda wrote: "Those [cells] which stain blue with haemotoxylin are large and open through the cuticle of the epithelium. They are apparently the mucin-secreting cells. In some preparations, however, a great many cells are almost devoid of slime, which was probably discharged while the worm was being narcotized with menthol.

"The cells staining with eosin appear to consist of two types, although this is not always evident. Those of one type, which stain red with eosin, though not very deeply, show a small nucleus at the base, are elongate and open through the cuticle. They are filled with granules. These cells are most common throughout all preparations studied. The cells of the other type are especially evident when Mallory's stain is used. They stain deeply with eosin. Under a high power of the microscope they are seen to contain fine granules and in some cells their content is homogeneous. I assume that these cells are merely the young, unripe ones of the second type.

"I believe that all the cells which stain in eosin are the light cells of the worm. It is interesting to note that the tip of the head of the worm, where no light appears, as already stated, shows none of the eosinophil cells at all." Photographs of the sections are reproduced as Fig. 54.

This structure suggests an external secretion but Kanda (1939) was unable to observe any luminous material on his fingers when the ani-

mal was strongly rubbed and the brilliant light appeared. Despite the fact that no luminous material separated, the gland cells do have ducts which open through the cuticle. Kanda has suggested that the luminous material may be small in amount and the light production take place in the ducts or at the moment of discharge, since the duration of luminescence is always short.



FIG. 54. Section of the skin of *Embletonema kandai*, showing mucous cells (m.c.) and luminous cells (l.c.). After Kanda.

Physiology and Biochemistry

If *Embletonema* is suddenly extended between two hands, the head end in one hand and the tail end in the other, the brilliant light appears over the whole surface of the long body except the tip of the head. Kanda found that chemical stimuli, like dilute acid or dilute alkali or dilute H_2O , also evoke a bright light. Adding excess $CaCl_2$ to the sea water caused rhythmic flashing of the worm, like a fire-fly, and in addition a faint and continuous glow.

The effect of pure solutions of various salts in 0.5 *m* concentration was also tested. When whole worms were placed in pure 0.5 *m* $NaCl$, $MgCl_2$ and $(NH_4)_2SO_4$, no light appeared, but a bright light was observed in 0.5 *m* KCl , $CaCl_2$ and Na_2SO_4 and a fainter one in 0.5 *m* NH_4Cl . $MgSO_4$ caused flashing after a certain time interval. If left too long in these solutions the worms were killed. Addition of

fresh water or saponin also caused luminescence, as did induced electric shocks and raising the temperature of the water to 32–33°C or cooling it to 1°C.

When a nemertean was placed on blotting paper, it luminesced brightly and when dried over P_2O_5 , the dried material again luminesced if moistened with water. Kanda found that the luciferin-luciferase reaction could not be demonstrated, whether the living worm or the dried material was used in the preparation. He was also unable to obtain luminescence on adding methyl or ethyl alcohol extracts of fresh or dried nemerteans to cold water or hot water extracts of the animal and wrote: "The cold water extract of the nemertean gives no light with *Cypridina* luciferin, nor does the hot water extract of the nemertean give light with *Cypridina* luciferase."

Thinking that the light might be due to symbiotic luminous bacteria, Kanda tested the effect of KCN, which diminishes the luminescence of bacteria in low concentrations, but was unable to find any inhibition by KCN even in fairly high concentrations. *Emplectonema* is undoubtedly self luminous. The effect of lack of oxygen was not tested and other gaps in our knowledge of nemertean luminescence indicate the need for further experimentation.

ROTATORIA

The only rotifer that has been described as luminescent is *Synchaeta baltica* from the Baltic Sea, observed by Michaelis (1830), together with other "infusoria" which were responsible for the luminescence in the harbor of Kiel. Michaelis called it a *Vorticella*. However, Ehrenberg (1834) was never able to observe luminescence in this form, although the animal was present in the first and later samples of sea water from Kiel sent him by Michaelis. Ehrenberg gave the new name, *Synchaeta baltica*, sp. nov., and published a beautiful figure of the animal (together with the luminous annelid, *Polynoë fulgurans*) in his great work, "Das Leuchten des Meeres." No other instances of luminous rotifers are on record, and Michaelis probably confused the light from dinoflagellates or worms with luminescence of *Synchaeta*. However, many writers on bioluminescence have continued to cite both Michaelis and Ehrenberg as observing luminous rotifers. Gadeau de Kerville (1890) thought the light might be due to luminous bacteria.

POLYZOA (BRYOZOA ECTOPROCTA)

The Bryozoa were formerly considered a phylum, divided into the Ectoprocta and the Endoprocta. The latter, now called Kamptozoa,

are minute, sessile, stalked animals living in the sea or in fresh water. Only about 40 species of endoprocts are known, none of which are luminous.

The members of the Bryozoa ectoprocta or the phylum, Polyzoa, the corallines or moss animals, live as colonies encrusting seaweeds, rocks, and piles. Some 1800 species have been described. Together with the hydroids and tunicates such forms were spoken of as zoophytes—plant-like animals—by early naturalists. They might be expected to emit light and there are quite a number of records of luminescence in the older literature. However, many motile luminous animals live among the polyzoa and frequently sessile luminous hydroids and polyzoa live close together covering a surface in an indistinguishable pattern. It is easy to mistake luminescence of a hydroid for that of a polyzoan.

The oldest observation, according to Ehrenberg (1834), was made by Peron (1804), who examined the sea floor near Cape Leuwin in New Holland and found "de Retepores, de Sertularies, d'Isis, de Gorgonies, d'Alcyons, et d'Eponges" to emit light. This description is too general to be considered a bona fide claim to discovery of the luminescence of Retepora, one of the Polyzoa.

The most striking observations are those of the Reverend Landsborough (1842), who collected specimens of the seaweed *Fucus* and noted that the luminescence which appeared on stroking the leaves came from the hydroids growing on them, *Laomedea geniculata* or *Sertularia pumila*, and also from the Corallines, *Flustra membranacea* (now *Membranipora membranacea*), and *F. pilosa* (now *Electra pilosa*). *Membranipora stellata* Thompson and *Valkeria geniculata* were also luminous. *Flustra* (*Membranipora*) *membranacea* was "very beautiful . . . it exhibited, when shaken, a simultaneous blaze and became, for a little, like a sheet of fire." Landsborough's descriptions of his observations are not too convincing and it is surprising that no more recent records are available. Hincks,¹ Gadeau de Kerville (1889), Mangold (1910), and most later writers quote Landsborough as authority for luminous polyzoa. Gadeau de Kerville also mentions *Scrupocellaria reptans* as luminous, without giving a reference.

Pelvet (1867), in describing the "phosphorescence de rivage" at Arrormanches, notes the luminescence of sertularians, campanularians, tunicates, annelids, crustaceans and "bryozoaires of the genus *Flustres*."

Finally, Verlain (1877) during a trip to the Isles St. Paul and

¹ A History of the British Marine Polyzoa (2 vols.). London, 1880, p. CXXXV of the Introduction.

Amsterdam in the Indian Ocean recorded finding a luminous *Bugula* in a cave whose walls were carpeted with sponges, ascidians, actinians, and bryozoans. The phosphorescence was noticed from a specimen at night after putting out the candle in his laboratory. He stated in a footnote (p. 67), "When I agitated the water I saw immediately all the branches of the bryozoan illuminated with living colors, changing instantly, passing with an astonishing rapidity from red to green or sky blue; but the phenomenon lasts only a short time and ceases when the water is no longer agitated."

The only record of the present century is that of Molisch (1912), who was particularly interested in determining whether marine algae were self-luminous or whether the light was always due to animals on them. At Helgoland, he convinced himself that *Membranipora pilosa* (now *Electra pilosa*), growing on various algae, was luminous and also referred to the work of Landsborough as showing the same thing. The author has been unable to observe luminescence of incrustations of *Electra pilosa* or *E. hastingsae*, growing on *Laminaria* fronds at Woods Hole, Massachusetts, either by electrical stimulation or by immersion in distilled water. There may be luminosity of some Polyzoa, but nothing is known of the distribution of luminosity in this phylum or of the structure or physiology of the luminous cells. Such an investigation and a re-examination of reported luminous species is much to be desired.

CHAETOGNATHA

This group of arrow-worms or *Sagittae* is one of the smallest phyla of the animal kingdom, comprising some thirty species distributed in six genera. They are relatively small animals, shaped like an arrow and completely transparent. Their distribution is wide among the plankton of all seas, from the surface to great depths.

Often associated with such transparent luminous forms as jelly-fish and comb jellies, there is every reason to expect luminescent species and two reports do occur in the literature, one by Giglioli (1870) and one by Khvorastansky (1892). Giglioli observed one luminous *Sagitta* in the Atlantic and another in the port of Anjer, Java. He described the light as feeble and coming from the posterior end near the tail. Other non-luminous forms were observed in the Gulf of Petceli off the North China Coast and the South Atlantic.

Khvorastansky's luminous specimen, *Sagitta bipunctata*, was obtained in the White Sea, the strait of Anser, in July 1890. The body of the animal luminesced with "une lumière claire" but only when alive, not when dead. The light came from the narrow part of the

back near the tail as described by Giglioli. Placed in alcohol the light disappeared instantly. Khvorastansky endeavored to find a luminous organ by making sections but observed nothing unusual in structure and merely supposed that the light came from epidermal cells.

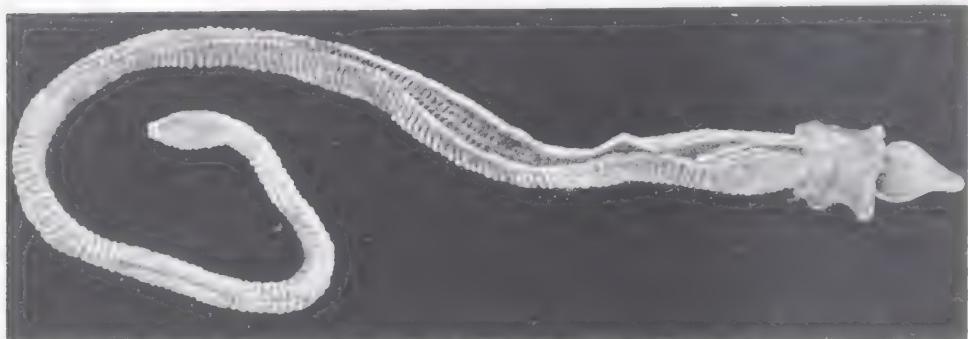


FIG. 55. The balanoglossid, *Ptychodera bahamensis*, from a drawing of Van der Horst, supplied by W. J. Crozier.

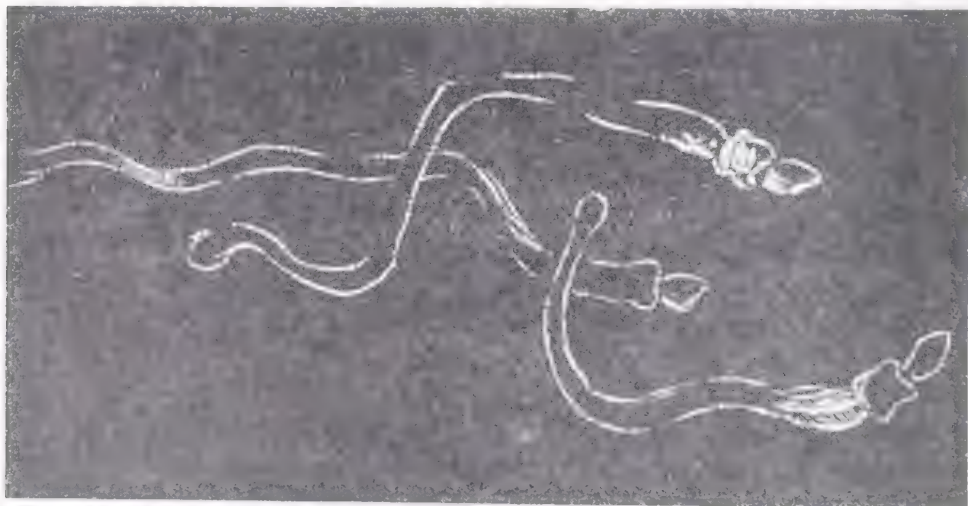


FIG. 56. *Ptychodera bahamensis*, showing the luminous regions, from a drawing by Van der Horst, supplied by W. J. Crozier.

ENTEROPNEUSTA OR HEMICORDATA

Panceri (1875) appears to have been the first to observe luminescence of these remarkable marine animals, the balanoglossids, shown in Figs. 55 and 56. Such earlier students of the group as Della Chiaje, Keferstein, Kowalewski, and Willemoes Suhm overlooked the light production. Balanoglossids are worm like forms, living in sand, frequently under rocks, with a long tongue like proboscis whose end

resembles an acorn—in Greek, *balanus* an acorn and *glossus*, a tongue. They are mostly shallow water inhabitants, although they have been dredged at such great depths as 2,500 fathoms. All have a disagreeable odor which can best be described as that of iodoform in the species the author has investigated.

The Enteropneusta are regarded as chordates by some and as a separate phylum, allied to the phoronids by others. Only 58 species and 12 genera are known, classified by C. Dawydoff² in 4 families, as follows:

Ptychoderidae (*Ptychodera*, *Balanoglossus*, *Glossobalanus*)

Harrimaniidae (*Dolichoglossus* or *Saccoglossus*, *Harrimania*, *Stereobalanus*, *Xenopleura*)

Glandicipitidae or *Sphengelidae* (*Schizocardium*, *Glandiceps*, *Spengelina*, *Willeya*)

Protoglossidae (*Protoglossus* or *Protobalanus*)

The underlined genera in the above list are known to be luminescent and some others may also emit light, but have never been investigated. *Dolichoglossus kowalevski* Agassiz, common at Woods Hole, Massachusetts, is not luminous. Molisch (1912) has recorded seeing the light of *Balanoglossus* at Trieste in 1902, and a Bermuda form, *Ptychodera bahamensis*, has been studied by Crozier, Dahlgren, and Harvey.

Histology

Panceri (1875) described *Balanoglossus minutus* from Naples in his paper on the luminous Anellidi. Although he considered it related to the Nemertinea the emission of light was very similar to that of the annelid *Chaetopterus*, a mucous from the surface epithelium. Among the ordinary epithelial cells there were bottle-shaped ones swollen with yellow material that looked like fat. These he regarded as the photogenic cells which disgorged their contents into the sea water.

Dahlgren (1917) has studied the histology of *Ptychodera bahamensis* from material sent to him by Crozier. Sections reveal an epithelium with several types of gland cells below it, mucous cells staining in mucin stains and luminous cells staining black in iron hematoxylin. In addition there were two more kinds of cells, one full of colorless granules and the other appearing as if their contents had been dissolved out in process of preparation, or as if they had discharged their contents. The cells staining black in iron hematoxylin were designated the photogenic ones, since, according to Dahlgren, the luminous cells in other luminous animals characteristically take this stain.

² From "Traité de Zoologie" edited by P. Grassé, Vol. XI, p. 451, 1948.

Physiology

The luminous slime is only produced on handling or stimulating the animal, as Panzeri (1875) noted. He called the color of the light pale azure and particularly noticed that no "luminous currents," i.e., no luminous waves, passed over the surface like those of pennatulids. Placed in fresh water, the light of *Balanoglossus* became continuous, gradually decreasing in intensity.

Crozier (1929) has described the luminescence resulting from various kinds of stimulation as "a vivid greenish light from every portion of the body save the gills. When placed in a dark room during daylight hours, they do not exhibit their phosphorescence in response to mechanical stimulation of various kinds. And even at night, after they have been in darkness for some time, 5-minute illumination by the relative feeble light from a 25 watt tungsten filament, placed 15 ft. from the aquarium, was found to make it more difficult to elicit the phosphorescent response." When placed in a dark room in the early morning, six hours later Crozier could not observe a luminescence unless animals were strongly pressed or struck. "Induced shocks of moderate strength, however, did induce light production at any time, whether the *Ptychodera* had been in darkness or in light."

The author has observed *Ptychodera* in Bermuda and also the species at Naples, *Balanoglossus minutus*, which was studied by Panzeri. The luminous slime readily comes off on the fingers, an undoubted extracellular secretion. A distinction must be made between the weak reflex stimulation of balanoglossids studied by Crozier, where luminescence is inhibited by light, and the ability to luminesce on strong stimulation, such as on roughly handling or grinding the animal, which is not inhibited. Unlike the situation in ctenophores sunlight or artificial light has no effect on the accumulation of luminous material in either *Ptychodera* or *Balanoglossus*.

Biochemistry

Attempts have been made to demonstrate the luciferin-luciferase reaction in both *Ptychodera* from Bermuda and *Balanoglossus* from Naples without success (Harvey, 1926). Hot water extracts of the balanoglossids, allowed to cool, that should contain luciferin, even when prepared in absence of oxygen, emit no light when mixed with cold water extracts allowed to stand until the luminescence disappears. Neither will *Cypridina* luciferin luminesce when mixed with a cold extract of balanoglossids nor *Cypridina* luciferase when mixed with a hot water extract of balanoglossids which should contain luciferin.

The author (1926) has also studied the dependence of luminescence of *Balanoglossus minutus* on oxygen. When the whole animal is shaken in sea water through which pure H_2 is passing, no luminescence can be observed, but on admitting air both *Balanoglossus* and sea water luminesce. In this respect *Balanoglossus* behaves like the majority of luminous forms where light production is dependent on dissolved oxygen. No special fluorescence of the slime or the epithelium of *Balanoglossus* can be observed in ultraviolet light.

CHAPTER VII

Annelida

INTRODUCTION

The great phylum Annelida, divided into the four classes, Archiannelida (primitive worms), Polychaeta (marine worms), Oligochaeta (earthworms) and Hirudinea (leeches), contains a fair number of luminous forms, restricted to the polychaetes and the oligochaetes. Among other characters, segmentation of the body, with paired appendages on each segment and a closed blood vascular system characterize this phylum. Annelids are found in both marine and fresh water or on the land. More than 6,500 species are known.

Luminescence has appeared in marine and terrestrial forms in at least 11 of the 76 families. The classification of polychaetes by E. Hempelmann and of oligochaetes by W. Michaelsen showing the distribution of luminous families (in italics) will give a good idea of the great variety of true worms.

Annelida

Archiannelida (5 families, 11 genera)

Polychaeta

Errantia

Amphinomidae (12 genera)

Aphroditidae (51 genera)

Chrysopetalidae or

Palmyridae (4 genera)

Pisionidae (2 genera)

Phyllodocidae (23 genera)

Alciopidae (8 genera)

Tomopteridae (2 genera)

Typhloscolecidae (3 genera)

Hesionidae (19 genera)

Syllidae (24 genera)

Sedentaria

Ariciidae (6 genera)

Spionidae (14 genera)

?Nereidae or *?Lycoridae* (7 genera)

Nephtyidae (1 genus)

Sphaerodoridae (2 genera)

Glyceridae (6 genera)

Eunicidae or *Leodicidae* (24 genera)

Histiobdellidae (2 genera)

Ichthyotomidae (1 genus)

Myzostomidae (4 genera)

Protomyzostomidae (1 genus)

Mesomyzostomidae (1 genus)

Stelecopidae (1 genus)

Arenicolidae (2 genera)

Maldanidae (27 genera)

Magelonidae (1 genus)	Oweniidae (2 genera)
Disomidae (2 genera)	Sternaspidae (1 genus)
Paraonidae (2 genera)	Amphictenidae (5 genera)
<i>Chaetopteridae</i> (7 genera)	Ampharetidae (15 genera)
<i>Cirratulidae</i> (13 genera)	<i>Terebellidae</i> (31 genera)
Chlorhemidae (4 genera)	Sabelariidae (9 genera)
Scalibregmidae (8 genera)	Sabellidae (24 genera)
? <i>Opheliidae</i> (9 genera with ? <i>Polyopthalmus</i>)	Serpulidae (23 genera)
Capitellidae (13 genera)	

Oligochaeta¹

Aelosomatidae (3 genera)	Syngenodrilidae (1 genus)
Naididae (13 genera)	Moniligastridae (4 genera)
<i>Enchytraeidae</i> (20 genera)	Glossoscolecidae (17 genera)
Tubificidae (15 genera)	Sparganophilidae (1 genus)
Phreodrilidae (4 genera)	Microchaetidae (7 genera)
Lumbriculidae (14 genera)	Hormogastridae (1 genus)
Branchiobdellidae (9 genera)	Criodrilidae (1 genus)
Acanthobdellidae (1 genus)	<i>Lumbricidae</i> (8 genera)
Phreoryctidae (2 genera)	<i>Acanthodrilidae</i> (40 genera)
Alluroididae (1 genus)	Eudrilidae (32 genera)
	<i>Megascolecidae</i> (17 genera)

Hirudinea (8 families, 87 genera)

POLYCHAETA

The discovery of luminous marine polychaetes can probably be attributed to Auzout and de la Voie in 1666 who both observed worms on oysters which "twinkled like a great star." In fact they described four kinds of worms, only some of which were luminous. *Terebellids* are commonly found on oysters today, and these men no doubt observed a *Polycirrus* or *Thelepus*. Nearly a century elapsed before the next records, the simultaneous discoveries of Vianelli in 1794, Grisellini in 1750 and the Abbé Nollet in 1750 of worms called *Scolopendra marina*, in the canals of Venice. According to Panceri (1878) they were syllids or nereids. Among other early naturalists who described luminous annelids were Adler in 1752, Baker in 1753, Le Roy in 1754, Baster in 1757, Forskal in 1762, Fougereux de Bonderoy in 1767, Fabricius in 1780, Spallanzani in 1798, Vivani (1805), Müller (1806), Tilesius (1819), MacCulloch (1821) and Ehrenberg (1834). These men were mostly interested in the phosphorescence of the sea, and their work, together with that of Quatrefages (1843) and de Filippi-Lessona (1864), definitely established the polychaetes as one source of sea light. It is practically impossible to identify the species which they observed. The small luminous worm called by Ehrenberg

¹G. E. Pickford has listed 15 families and additional genera.

(1834), *Polynoë fulgurans*, from the waters of Kiel harbor, is reproduced as Fig. 57.

Quatrefages, a specialist on annelids, was the first to pay special attention to luminescence. He noticed that the Fucus at Isle Chausey near St. Malo, France, was brilliantly luminous, due chiefly to small annelids and ophiurans crawling on it. Under the microscope the worms were observed to have two rows of luminous spots on the segments, looking like little stars of light. Luminescence only appeared when the muscles contracted, leading Quatrefages to disagree with Ehrenberg, who had spoken of a light organ in annelids, and to hold that the light of both annelids and ophiurans came from the muscles.



FIG. 57. *Polynoë fulgurans*, as figured by Ehrenberg in 1834.

The modern work, as in the case of so many other luminous animals, may be said to begin with Panceri (1878) who investigated many species of polychaetes, belonging to five families. Actually seven or eight² different families of marine worms contain species now known to produce light. The method of lighting and the luminous organs differ greatly and will be considered under separate headings. An idea of the various luminous (in *italics*) and non-luminous genera can be obtained from the following table based on the F. Hempelmann classification:

Families of Polychaetes Containing Luminous Genera

Errantia

Aphroditidae

Hermioninae (Hermione, Aphrodite, Laetmonice, Pontogenia)

Polynoinae (Macellicephala, Lepidonotus, Euphione, Malmgrenia, Gattviana, Perolepis, Eunoe, *Harmothoe*, Herdmanella, Scalisetosus, Hololepidella, Lagisca, *Polynoë*, Polynoella, Enipo, Drieschia, Nemidia, Melaemus, Halosydna.

² In the article on Polychaeta by W. B. Benham in the Cambridge Natural History (1901), the suggestion is made that the "segmental eyes" of Polyphthalmus of the family Opheliidae may be luminous organs, but light emission of this worm has not been observed.

Gastrolepidia, Hemilepidia, Acanthicolepis, *Lepidasthenia*, Nectochaeta, Allmaniella, *Acholoë*, Lepidametria, Hyperhalosydna, Halosydnoidea, Polynoëlla, Iphione, Bylgia, Admetella)

Acoetinae (Polyodontes, Panthalis, Eupanthalis, Restio, Eupolyodontes)

?*Sigalioninae* (Sigalion, Psammolyce, Sthenelais, Euthalenessa, Leanira,

Sthenolepis, ?*Pholoë*, Eulepis, Peisidice)

Alciopidae (Asterope, Alciopa, Vanadis, Greefia, *Corynocephalus*, ?*Rhynchonerella*, *Calizonella*, Calizona)

Tomopteridae (*Tomopteris*, *Enapteris*)

Syllidae

Syllinae (Syllis, Trypanosyllis, Eurysyllis, Xenosyllis, Opisthosyllis)

Eusyllinae (Opisthodonta, *Odontosyllis*, Fauvelia, Pterosyllis, Streptosyllis,

Syllides, Pseudosyllides, Spionosyllis, *Pionosyllis*, Parapionosyllis, *Eusyllis*)

Exogoninae (Grubea, Sphaerosyllis, Exogone, Spermosyllis)

Autolytinae (Autolytus, Myrianida, Virchowia, Procerastea)

?*Nereidae* or *Lycoridae* (Lycastis, Micronereis, Leptonereis, ?*Nereis*, ?*Perinereis*, Ceratocephale, Dendronereides)

Sedentaria

Chaetopteridae (*Chaetopterus*, *Mesochaetopterus*, Ranzania, *Telepsaurus*, Spirochaetopterus, *Phyllochaetopterus*, *Leptochaetopterus*)

Cirratulidae

Cirratulinae (Audouinia, *Cirratulus*, *Heterocirrus*, Tharyx, Chaetozone, Dodecaceria, Acrocirrus, *Macrochaeta*, Streblospio = Hekaterobranthus, Cirrophorus)

Ctenodrilinae (Ctenodrilus, Zeppelinina, Raphidrilus)

Terebellidae

Artacaminae (Artacama)

Amphitritinae (18 genera)

Thelepininae (Streblosoma, *Thelepus*, Euthelepus, Parathelepus)

Polycirrinae (*Polycirrus*, Ereutho, Amaea, Lysilla, Hauchiella)

Trichobranchinae (Trichobranthus, Octobranthus)

Canepharinae (Terebellides)

Aphroditidae, Polynoinae

General. Although Quatrefages (1845) undoubtedly observed polynoid worms, the best account of the light has been given by Panceri (1898) who studied *Polynoë lunulata* and described the new species, *Polynoë turcia* and *Pholoë brevicornis*. In this subfamily luminescence is restricted to the scales, a pair to a segment, which cover the dorsal surface in two imbricated rows, like shingles on a roof, shown in Figs. 58 and 59. Panceri noted that the luminous region might cover the entire scale (except for point of attachment) or a half-moon-shaped area of light, depending on the species. The light appeared only on stimulation and might proceed along the scales from head to tail or tail to head.

The general lighting behavior of a polynoid worm has been well described by Haswell (1882), who wrote as follows: "When certain

species of *Polynoe* are irritated in the dark a flash of phosphorescent light runs along the scales, each being illuminated with a vividness which makes it shine out like a shield of light, a dark spot near the centre representing the surface attachment where the light producing tissue would appear to be absent. The irritation communicates itself from segment to segment, and if the stimulus be sufficiently powerful,



FIG. 58. The luminous polynoid worm, *Acholoë astericola*. After Kutschera.

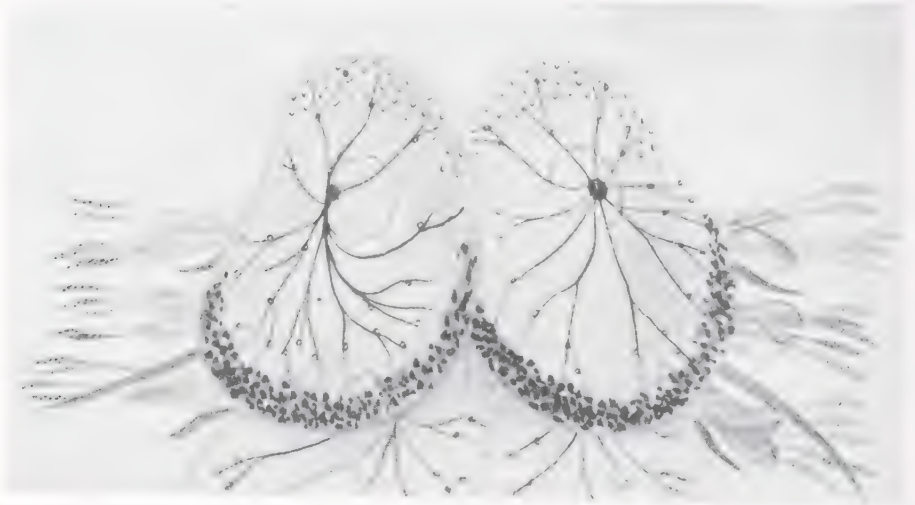


FIG. 59. Elytra from one segment of *Acholoë astericola*. Oval area indicates point of attachment, from which nerves can be seen branching over the scale toward the "teeth" (dots at top) and what Kutschera regarded as light organs (semicircle of densely crowded dots or papillae below). After Kutschera.

flashes of phosphorescence may run along the whole series of elytra, one or more of which then become detached, the animal meanwhile moving away rapidly and leaving behind it the scale or scales still glowing with phosphorescent light. The species in which the phenomenon of phosphorescence occurs are species characterized by the rapidity of their movements, and also by the readiness with which the scales are parted with; and it seems not at all unlikely that the phos-

phorescence may have a protective action, the illuminated scales which are thrown off distracting the attention of the assailant in the dark recesses which the Polynoidae usually frequent." Kutschera (1909) also believed the purpose of the light was "Schreckwirkung."

Luminous polynoid worms are found in both tropic and arctic waters. Khvorostansky (1892) has described *Polynoë aspera* from the White Sea and Lloyd (1907) found *Lepidasthenia stylolepis*, n. sp., at 25 fathoms at the head of the Persian Gulf. He wrote: "Before dropping the worm into the (formalin) solution, no phosphorescence was noted, but under the influence of the irritant, two rows of brilliant points of light, one on each side of the animal's back, became visible, and remained so for several seconds before gradually fading away."

Histology. The initial view regarding photogenic cells must be attributed to Quatrefages (1843) who erroneously thought muscles were the source of light. A second view came from Panceri (1878), who was led astray in his designation of the nerve endings in *Polynoë* as the source of light because of the superabundance of nerve fibers in the elytra, which he thought disproportionate for other possible functions.

This mistake was rectified by Jourdan (1885), who first prepared serial sections and suggested that secretory cells of the hypodermal layer on the under surface of the scale in the luminous species, *Polynoë orquata*, functioned as photogenic cells. They were fundamentally similar to mucus secreting cells of the non-luminous species, *Polynoë grubiana* and are shown in Fig. 61. In Figs. 60 and 61 is shown a section of the chitinous papilla on the dorsal surface of a scale of the non-luminous *Polynoë grubiana* showing ganglion cells and nerve fibers going to sensory receptors in what Jourdan regarded as a sense organ.

No further histological study of luminous polynoids was made until Kutschera's (1909) extensive work. Curiously enough Kutschera came to a fourth and quite different conclusion regarding the photogenic cells in *Acholoë astericola*, a polynoid worm living in the ambulacral grooves on the under surface of the arms of the sea-star, *Astropecten auranticus*. In this species the light comes from all parts of the scale except in those animals whose ability to luminesce was weakened, when only a half moon-shaped region of the posterior margin becomes luminescent. This luminous area is studded with brown pigment and light organs are represented by the cuticular papillae, shown in cross section in Fig. 60. Kutschera found, at the base of the papilla, gland cells arranged like a star, which were supposed to secrete a luminous material into the sea water through a canal and pore.

Although Kutschera could observe no external luminous secretion

when scales were touched, he nevertheless believed the luminescence was extracellular and appeared when the secretion touched the sea water. Ordinarily secretion resulted from nerve stimulation, but for rapid emptying of the glands, contraction of muscles of the elyptrophore

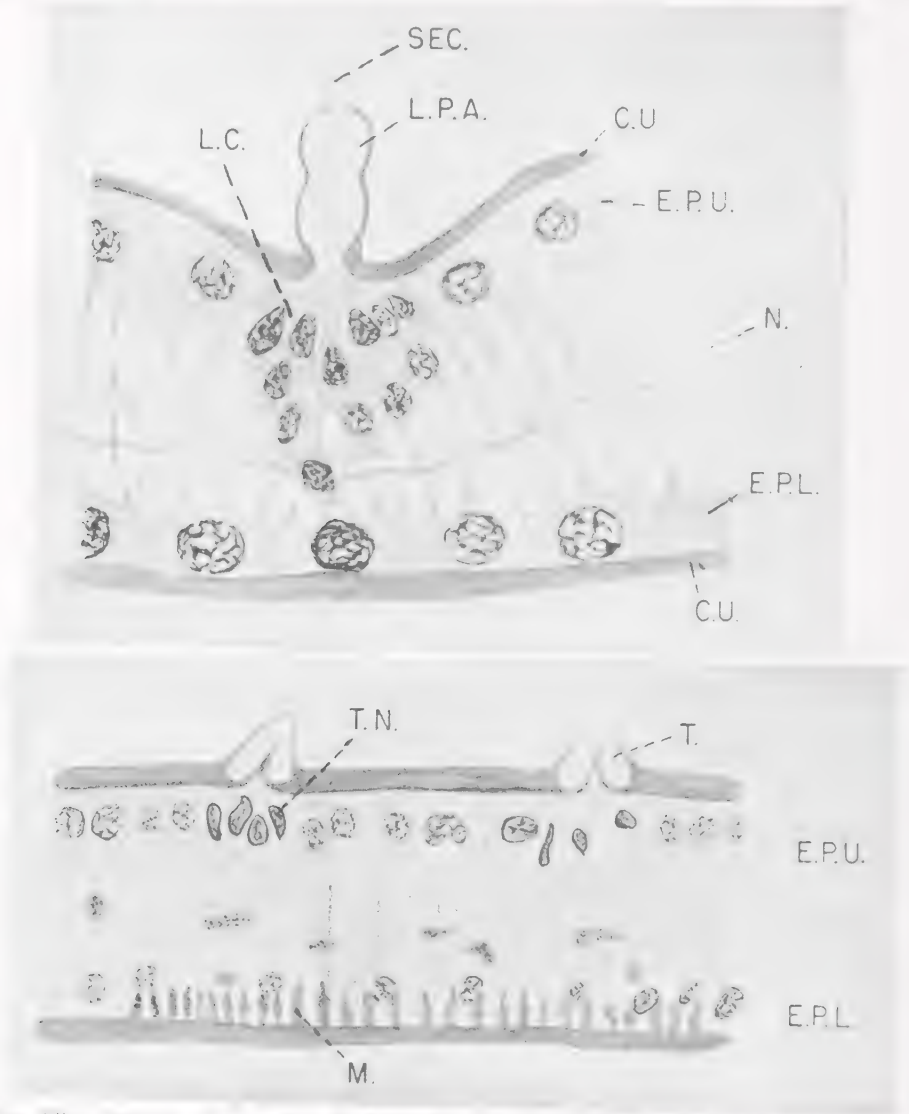


FIG. 60. Section of a scale of *Acholoe* showing papilla (above), and tooth (below) with luminous cells (L.C.); L.P.A., light papilla; SEC., secretion; C.U., cuticle; E.P.U., upper hypodermis; E.P.L., lower hypodermis; N., region of nerve distribution; T.N., tooth nuclei. After Kutschera.

and perhaps also of connective tissue fibers in the scale might be involved. In addition to the luminous papillae there were also mere tooth-like projections with few underlying cells, on the anterior margin of the scale. Their function was not determined but presumably was

sensory. Dahlgren (1916) figured the tooth-like projections and the papillae of *Lepidonotus squamata* and was inclined to interpret light production as did Kutschera.

The latest study of the scales of luminous polynoid worms, by Bonhomme (1940, 42), has resulted in a modification of Jourdan's view. Bonhomme studied two forms, *Harmothoe impar*, with a large luminous crescent on each scale and particularly, *Polynoë lunulata*, with a very small crescent. Figure 61 shows the luminous areas. He believed that Kutschera's photogenic cells at the base of the papillae are ganglion cells and that the papillae are sense organs. Under the microscope he could see no external secretion on the scales. However, the pigment cells and papillae stood out clearly due to the emission of light by that group of cells on the ventral surface of the scale designated

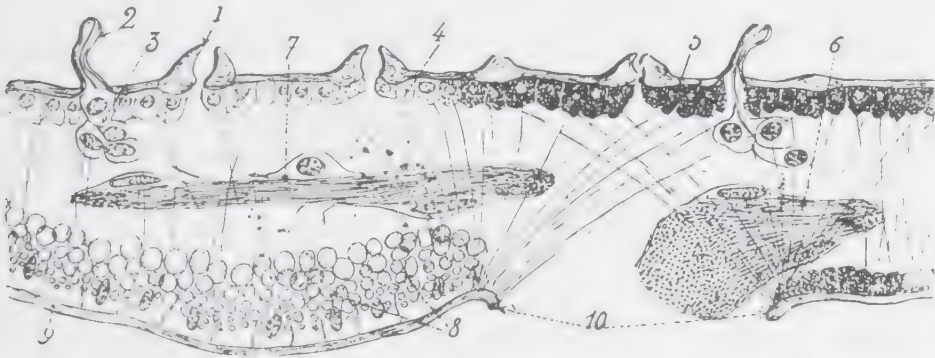


FIG. 61. Section of a scale of *Polynoë lunulata* showing tooth (1), papilla (2), sensory cells (3), and luminous cells (8), according to Bonhomme.

as photogenic by Jourdan. However, Bonhomme believed that the light was intracellular.

He described minutely these "photocytes," which have rather large elliptical nuclei and are packed with large secretion granules ("grains"), deformed by their closeness to each other. Tests with osmic acid, Soudan III, and Scharlach R, indicated that the granules were not fat. They did not stain with mucous stains but did give a protein test and he suggested that they might be a protein-substrate combination. Bonhomme was not certain whether phosphorus or oxidase were present.

After continued stimulation there was no indication of loss of granules, a fact that clearly supports the idea of intracellular luminescence. Many nerve fibers, stained by the silver nitrate technique of Bielschowsky, were seen to ramify at the level of the photocytes and the behavior of isolated scales as compared with the whole animal was

intimately connected with the nerve connection, as described under the section on physiology.

Physiology. All observers agree that polynoid worms light only on stimulation of various kinds, mechanical, chemical, thermal, electrical, etc. Although Panceri (1878) stated that scales which were detached did not light, he probably referred to spontaneous luminescence, for these organs readily luminesce on stimulation.

The work of Falger (1908) and Kutschera (1909) on *Acholoë astericola* consisted largely of an analysis of the types of excitations mentioned above and the results of both men will be combined in the following account. Placed in a dish of sea water, *Acholoë* is continuously on the move rapidly creeping along the surface of the glass and endeavoring to climb the side walls. At this time the greenish blue light may appear spontaneously sometimes at front or hind end, sometimes in the middle, and occasionally a single scale will light up with a weak glow. If one part of a worm is touched with a needle the light spreads from the region touched. According to Falger, the spread is either forward or backward, lasting a few seconds and then disappearing in some scales before others. According to Kutschera the conduction of the light wave is always from front to rear, not the reverse. When an animal is cut in half only the tail end lights and the front end crawls away dark. Perhaps the condition of the animal may explain the conflicting statements of Falger and Kutschera regarding direction of movement of the light wave. On each scale the luminescence is intermittent, that is, it may flicker twenty to thirty times in several seconds and may light uniformly or partially in the frequently described half-moon-shaped area.

Falger's chief work was concerned with electrical stimulation, both the constant galvanic current and induced faradic shocks. For these experiments the worms were placed on a glass plate and connections made by tinfoil strips at front and hind ends. Using a constant current from 3-volt batteries Falger observed the whole animal to light on the make, remain nearly dark during passage of the current, and again light on the break. There appeared to be no relation between the point of origin of the light and the direction of the current. With the interrupted induced current, the light appeared bright and constant at first but later became weaker and flickering and might last for twenty to thirty minutes. When exhausted by long stimulation a worm would recover after some hours in sea water. Isolated scales could be stimulated to luminesce electrically just as the whole animal. On continued stimulation the light might last for thirteen minutes, and if placed in sea water the scale would recover the ability to light on stimulation.

From excitation of isolated scales, Kutschera attempted to observe under the microscope isolated points of light that might represent the papillae or actual secretion of luminous material, but despite many trials was unsuccessful. He observed only a homogeneous illumination, beginning in the half-moon-shaped region and in vigorous animals spreading over the whole scale. All attempts to see a minute mass of secretion poured into sea water failed, and no secretion could be wiped off on filter paper. Therefore no experimental proof exists that the luminescence of polynoid worms is an external secretion, and the evidence seems to be against the papillae as luminous organs and definitely in favor of Bonhomme's intracellular luminescence of a photocyte layer near the under surface of the scale.

The papilla is probably a receptor organ sending nerve impulses to the ventral central ganglion chain which then returns them to the photocytes along nerves clearly seen to innervate that tissue.

Biochemistry. Little is known of the chemistry of light production. Bonhomme's microchemical tests already mentioned indicate the photogenic granules are not fat. The process is undoubtedly an oxidation. Falger (1918) noted that when pure oxygen was bubbled through sea water for thirty minutes, the luminescence of the worm on stimulation was very bright. In sea water bubbled with CO_2 for the same time, the light was about the same as in sea water. In order to remove oxygen completely Falger boiled sea water, cooled it away from the air, and bubbled CO_2 through it for $1\frac{1}{2}$ hours. Worms introduced into this deaerated but CO_2 -saturated sea water did not light on stimulation but were capable of lighting when returned to sea water.

The use of CO_2 to render water oxygen-free is not a faultless method, and the author (1926) has used pure hydrogen as a neutral gas to remove oxygen. The experiments established without doubt the fact that oxygen is necessary for luminescence in *Acholoë astericola*.

Attempts were also made to demonstrate the luciferin-luciferase reaction but without success. Even when the *Acholoë* "luciferin" solution was prepared in absence of oxygen, no light appeared when mixed with a cold water extract of the scales of this worm (*Acholoë* "luciferase"), and no light with *Cypridina* luciferase. *Cypridina* luciferin emitted no light in the presence of *Acholoë* luciferase.

When examined in ultraviolet light, some non-luminescing specimens of *Acholoë* were observed by the author (1926) to be brightly yellowish fluorescent over the scales and other specimens not. However, the latter, when stimulated to luminesce strongly, also show the yellowish fluorescence after the luminescence has subsided. Some waste product of the luminescent reaction is evidently highly fluores

cent and some of the worms, despite careful handling, must have luminesced previously. The non-luminous, *Polynoe grubiana*, exhibits only the bluish fluorescence characteristic of skeletal parts when its scales are examined (both upper and lower sides) in ultraviolet light. The setae are also brightly bluish violet fluorescent.

Alciopidae

Although Greeff (1885) described an alciopid from the Guinea islands, whose name, *Rhynchonorella fulgens*, suggests the ability to luminesce he made no mention of luminosity. The two references to luminescence of this family come from Lo Bianco (1909) and Okada (1925). Lo Bianco stated that in the male but not the female of *Calizonella lepidota* var. *Krohnii*, "The posterior region of each parapodium bore a large black pigmented structure which with great possibility was to be interpreted as a luminous organ." Apparently no observations of the luminescence have been recorded.

The experience of Okada has been more definite. He thought he had discovered the luminescence of a sponge, *Crateromorpha meyeri*, dredged from 1,000 meters on the bottom of the Sagami Sea but found that its light was really due to a small annelid belonging to the Alciopinae. Many of these annelids filled the canal system of the sponge and gave off "a thousand spots of a blue light resembling the stars in the sky. On dipping the sponge into fresh water the light shone particularly brightly, but at the same time the luminous spots were observed to be transferred from the body of the sponge into the surrounding medium." Nothing is known of the histology, physiology, or biochemistry of luminous organs in the Alciopidae.

Tomopteridae

These remarkable transparent pelagic annelids (see Fig. 62) were described by J. Müller in Oken's *Isis* in 1825 and also obtained by Quoy and Gaimard (1827), who referred to them as *Briarée scolopendre*. They are widespread in seas of all temperatures. The most noticeable feature of the otherwise colorless worms is a series of yellow structures, visible to the naked eye as yellow spots on each of the parapodia, which are large and adapted for swimming. These spots appear to have been first described by Busch (1847) without suggesting a function, and later by Vejdowsky (1878), who designated them as eyes. Greeff (1879) thought they were glandular organs of some kind and later observed their luminescence during his stay on the West African island of Rolas in 1880. Since then, the luminescence has been observed by many

workers, Kiernik (1908), Dahlgren (1916), Harvey (1926), Meyer (1930), and others.

There are two genera, *Tomopteris* and *Enapteris*, in the Tomopteridae. The number and distribution of luminous organs differ in different species. Greeff studied chiefly the morphology, and little is known of the physiology, except that light appears on stimulation. The author was unable to demonstrate the luciferin-luciferase reaction in *Tomopteris helgolandica* from Plymouth, England, and also found that cold water extracts of the luminous organs of the worm gave no light

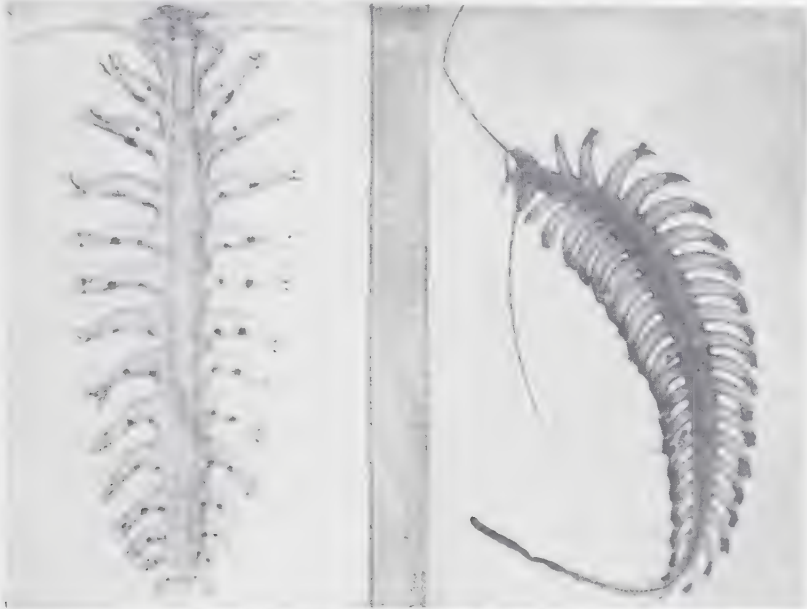


FIG. 62. The tomopterid worms, *Tomopteris rolosi* (left) showing the luminous organs as black dots (after Greeff) and *Tomopteris helgolandica*, photographed by H. P. Bigelow (after Dahlgren).

with *Cypridina* luciferin solution, nor did hot water extracts of the worm luminesce on addition of *Cypridina* luciferase. Nothing is known of the biochemistry of light production in *Tomopteris*, nor of the bright yellow pigment, so conspicuous in the photogenic cells. The color of the light is yellowish, but its spectral distribution is unknown.

Histology. No detailed histological study of the light organs has been made, but Greeff (1879, 82, 85) has described the organs from living specimens and published some diagrammatic drawings (see Fig. 63). There are present on the animal two types of light organ, large and small, and in addition "fin glands" (Flossendrüse) on the parapodia. The exact structure differs in different species but in general, there is a ring or rosette of yellow, presumably photogenic cells, ar

ranged like the segments of an orange, surrounded by a layer of elongated transparent cells, forming a sort of sheath. A nerve ganglion is to be found on the inner end of the large light organ, with fibers running between the transparent cells to the base of the yellow cells. The intensely yellow pigment is contained in a spherical droplet in each yellow cell. Luminescence is presumably intracellular, but further investigation is needed to establish this point. Meyer (1930) has

come to the conclusion that the luminous organs of *Tomopteris* are transformed nephridial funnels.

Syllidae

No worms present a more striking luminous display than the syllids, in which phosphorescence is intimately connected with mating reactions. In Venice, particularly, and in many other parts of the world the phosphorescence of syllid worms aroused the attention of scientists during the eighteenth century. Panceri (1878) has listed the various observers and he himself studied an *Odontosyllis* found near Posilipo, a suburb of Naples. The greenish light was "fulgentissima," making it easy to find the animal. The whole worm appeared like a

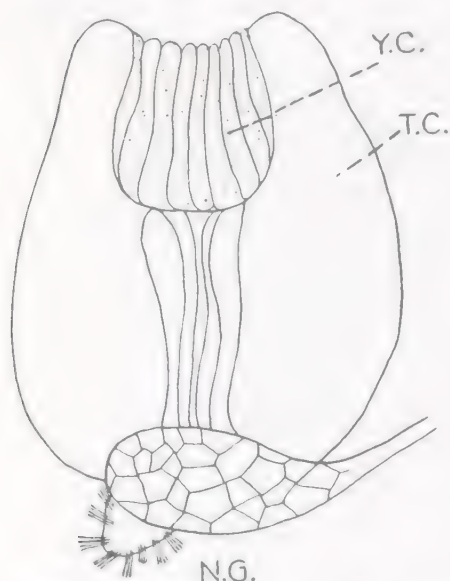


FIG. 63. Diagram of the large light organ of *Tomopteris mariana*, showing yellow cells (Y.C.), transparent cells (T.C.), and nerve ganglion (N.G.). After Greeff.

shining thread, and under the microscope Panceri could see lively scintillations, coming from minute luminous points and circular bright areas. When handled the body gave off a liquid luminescence which spread like a cloud in the sea water.

Swarming Reactions. A common characteristic of some syllids, nereids, and eunicids is the habit of swarming at certain seasons of the year, particularly in relation to phases of the moon. When the swarming is associated with displays of luminescence, as in syllid worms, the phenomenon becomes of popular interest, and the animals are often spoken of as "fire worms." The Bermuda fire worm, to be described in detail, is probably the best known, but others, all species of *Odontosyllis*, have been observed in Jamaica (Lund, 1911), the Bahamas (Crawshaw, 1935), the Pacific Coast (Potts, 1913; Fraser, 1915) and near Batavia, Java (Lummel, 1932).

It is possible that Columbus saw a display of fire-worms as he approached the new continent on his first voyage, as he described a light like "a taper in motion" as he approached the Bahamas. Crawshaw (1935) has made this suggestion from his study of the Bahaman species of *Odontosyllis*, observed at Abaco in 1921-23. The luminous display occurred in six out of seven observations within twenty-four hours of the moon's last quarter and rather long after sunset, when it was quite dark. In some months the worms were not observed, but they appeared in January, April, May, July, October, and December. "Half a dozen or 20 females will appear suddenly" emitting "a stream of brilliantly luminous matter . . . with the extruded ova, probably 2 or 3 times repeated at short intervals; and with this as a signal a number of males rush in, with short intermittent light flashes, toward the light for fertilization, partly with attachment to the female, partly darting rapidly too and fro through the luminous trail."

In Bermuda the swarming of *Odontosyllis enopla* usually occurs during a three- to five-day interval but sometimes for as much as eleven days, beginning about two days after the full moon during most months of the year, winter or summer. The greatest number of worms appear in the summer time. The connection with the moon's phases is very apparent. There is also a relation to the fading light. Records kept by Dr. E. L. Mark over a period of years during the summer, show that the first worms appear about fifty-five minutes after sunset.

There have been fewer observations in winter, but Huntsman (1948) has recently published data obtained from October, 1925, through March, 1926. In winter time the swarming and luminescence also began about fifty-five minutes after sunset and lasted about a half hour. However, the display varied at different months from one to as many as fifteen days after full moon, but always occurred before the moon had risen. Huntsman believed the tides were too small to account for the time of swarming and that it was related to a rapid decrease in light intensity from day-light to starlight (not moonlight).

The best description of the swarming in Bermuda has been published by Galloway (1908), who observed the worms during the summer of 1904 at the Flatts inlet to Harrington Sound, and later carefully described the anatomy and histology (Galloway and Welch, 1911). These authors wrote: "The display lasted from twenty to thirty minutes. Only a few worms appeared at first, each evening. The numbers gradually increased to a maximum, when scores might be seen at once. The display waned somewhat more rapidly than it waxed. An occasional belated specimen sometimes appeared some minutes afterward.

"The males and females differ considerably in size—the female often being twice as long as the males. The larger female specimens attain a length of 35 mm. Both sexes are distinctly phosphorescent, the female with strong and more continuous glow, and the male with sharper, intermittent flashes.

"In mating, the females, which are clearly swimming at the surface of the water before they begin to be phosphorescent, show first as a dim glow. Quite suddenly she becomes acutely phosphorescent, particularly in the posterior three-fourths of the body, although all the segments seem to be luminous in some degree. At this phase she swims rapidly through the water in small, luminous circles two or more inches in diameter. Around this smaller vivid circle is a halo of phosphorescence, growing dimmer peripherally. This halo of phosphorescence is possibly caused by the escaping eggs, together with whatever body fluids accompany them. At any rate the phosphorescent effect closely accompanies ovulation, and the eggs continue mildly phosphorescent for a while. The fact that the luminosity is known at no other time is further suggestive that it is produced by the material which escapes from the body cavity. If the phosphorescent glands are external, as the histology of the epidermis at least suggests, the discharge of the glands is closely correlated with ovulation.

"If the male does not appear, this illumination ceases after 10 to 20 seconds. . . .

"The male appears first as a delicate glint of light, possibly as much as 10 to 15 feet from the luminous female. They do not swim at the surface, as do the females, but come obliquely up from the deeper water. They dart directly for the center of the luminous circle and they locate the female with remarkable precision, when she is in the acute stage of phosphorescence . . . they rotate together in somewhat wider circles, scattering eggs and sperm in the water. . . . So far as could be observed, the phosphorescent display is not repeated by either individual after mating. Very shortly the worms cease to be luminous and are lost."

The phosphorescence of *Odontosyllis* is undoubtedly a mating adaptation serving to bring the sexes together at a time when eggs and sperm are ripe. The author has observed many displays in Bermuda and can vouch for the extraordinary accuracy of timing after sunset and the description of various observers.

A closely allied species, *Odontosyllis phosphorea*, was described by Moore³ from material which was labelled "Phosphorescent annelids caught at surface, Avalon Bay, Catalina Island, California; evening.

³ J. P. Moore, *Proc. Acad. Nat. Sci. Phila.*, **61**, 327, 1909.

April 11, 1903." The same species has been observed by Potts (1913) during June and July at the Biological Laboratory near Nanaimo on Vancouver Island, British Columbia. In this case also the worms live among rocks on the bottom and rise to the surface at the time of swarming, about 30 minutes after sunset, but the males and females, although brightly luminous, merely swim in wavy wiggles and apparently do not use the phosphorescence for mating. Potts could detect no apparent attraction between them as in the Bermuda and Bahama species. *Odontosyllis phosphorea* reaches the surface well before darkness begins.

Further observations were made by Frazer (1915), also at the Nanaimo Laboratory, who confirmed Potts' account of behavior. He could detect no relation to the phases of the moon in sporadic observa-

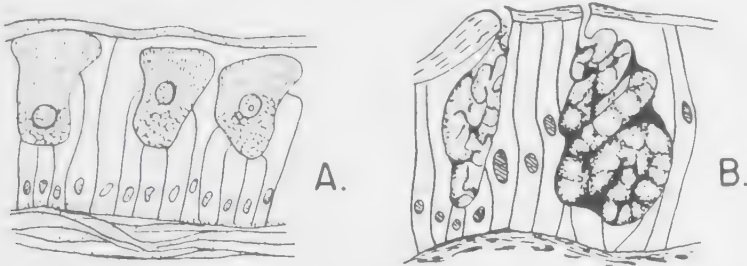


FIG. 64. Cells from the epidermis of *Odontosyllis enopla*, according to a drawing of Galloway and Welch, who labeled the B type phosphorescent (?).

tions made during June to December. The worms were few in June and December, but in other months they appeared, sometimes twice a month and always between sunset and dusk, irrespective of the actual clock time, indicating a definite relation to fading daylight. The males usually appeared first, followed by the females. When eggs and sperm had been shed, both sexes sank below the surface of the water.

The author has caught *Odontosyllis phosphorea* at Nanaimo at dusk, near the end of June, when there was an almost full moon. The worms did not luminesce when swimming or when handled but on crushing in a mortar a bright persistent greenish blue light appeared.

Histology. Galloway and Welch found two kinds of gland cells in the epidermis of *Odontosyllis*, the first rather large, of a common type in epithelia, and the second a flask-shaped variety with irregular surface depressions that gave the appearance of a spiral twist. Each cell had a distinct neck and opened to the exterior through a pore in the cuticle. Since these latter glands were often apparently empty, they might be the phosphorescent organs, "though of this the authors have no final proof." In the male, the "twisted" glands were also present.

of a slightly different structure. The two types are shown in Fig. 64.

Dahlgren (1916) was inclined to doubt that the twisted cells were photogenic and regarded them as mucous cells. Since the worm produced such an abundant secretion it seemed reasonable to look for rather large glands with a reservoir. These he found in fixed material on the ventral basal portion of the neuropodium, close to the nephridium. There were two or three kinds of cells present, distinguished by staining reactions with Delafield's hematoxylin and eosin. Some contained large light yellow non-staining granules. Their ducts were long and separate but contiguous, emptying at the tip of the dorsal lobe of the neuropodium. The whole arrangement,

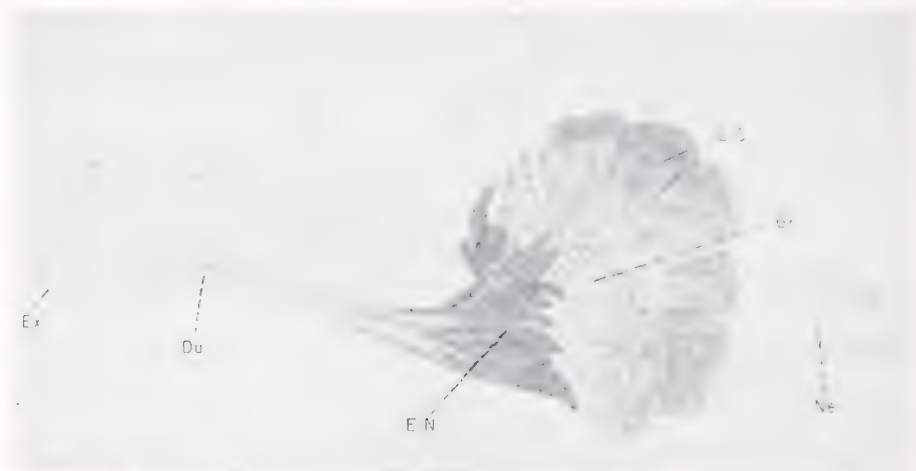


FIG. 65. Section of a parapodium of *Odontosyllis*, showing the large light gland with a duct, Du. LC, light cells with granular masses Gr; E.N., accessory cells of light gland; EX, outlet of duct; Ne, nephridium. After Dahlgren.

shown in Fig. 65, corresponds to what might be expected for the manufacture of a copious external secretion.

Biochemistry. In view of the external secretion and the structure of the luminous gland, with at least two types of cells, the presence of luciferin and luciferase was expected and in fact the author (1931) has been able to demonstrate the reaction, both with *Odontosyllis phosphorea* at Nanaimo, British Columbia, in 1920 and with *Odontosyllis enopla* at Bermuda in 1929.

To prepare luciferase, a worm was ground in a mortar and gave a brilliant bluish luminescence. When fresh water was added, the luminescent solution became brighter at first but lost its brilliance after some time, and the light disappeared. The luciferin solution was prepared by adding boiling fresh water to a worm and breaking up the animal. When the two solutions, both dark, were mixed, a good lu-

minescence appeared. It was not possible, however, to obtain light on mixing *Odontosyllis* luciferase with *Cypridina* luciferin or *Cypridina* luciferase with *Odontosyllis* luciferin.

The possible fluorescence of the material, the necessity of oxygen for luminescence and the general chemical properties of the luminous substances have not been investigated.

Nereidae

The knowledge of luminous Nereids is in a most unsatisfactory state, as it is difficult to identify the worms that were designated as *Nereis* by early workers. For example, Forskal in 1762 noted luminous *Nereis coerulea*, *N. pelagica*, and *N. viridis* in the Kattegat, Fabricius in 1780 a *Nereis noctiluca* from Greenland, and Viviani (1805) described *Nereis radiata*, *N. cirrhigera*, and *N. mucronata* in the Mediterranean. Some of these worms were probably syllids.

There appear to be no certain observations in recent times of any of the *Nereidae* producing light. Herdman (1903) described heteronereids and a peculiar phosphorescent phenomenon in the Gulf of Manaar, but only "suggested" the light came from nereids and the account is not convincing. Dr. Stanislaus Skowron has written (1928) the author that he observed the light of *Heteronereis* at Messina. Some individuals were luminous and some not, but no further details are available. It is quite possible that the nereids must be crossed off the list of luminous families.

Chaetopteridae

This family of bizarre sedentary worms, living in parchment-like tubes in the sand, is widely distributed and well known to most students of littoral marine life. The various segments of the body have been modified for different purposes and can be divided into an anterior portion, sometimes called the "head," a middle and a posterior portion, well shown in Fig. 66. The most unusual features are two feeler-like processes on the fused anterior segments, a pair of long wing-like processes at the beginning of the middle portion and the three "paddles" whose movement propels a stream of sea water containing food and oxygen through the tube. The morphology and histology have been thoroughly investigated by a number of workers, but many aspects of the biochemistry of luminescence remain unknown.

Despite the ease with which *Chaetopterus* can be kept in the laboratory, its lighting ability appears to have been observed first at Trieste by Will (1844), who noticed that a worm removed from its tube and placed in a dish would move on the bottom without lighting, but when

disturbed, a luminescent slime appeared. The intensity became less on continued stimulation. The slime was secreted by gland cells of flask or pear shape, sometimes forming polyhedral follicles (Balgen). The color of the light was described as greenish, and it is interesting to note that Ray Lancaster (1868), in one of the earliest spectral studies on luminous animals, examined "the phosphorescent mucus secreted by glands at the base of the large pinnules in the annelid, *Chaetopterus insignis* Baird. The light gave a diffused spectrum from about line 5



FIG. 66. *Chaetopterus variopedatus*, removed from its tube. Head end at lower left. Photo by E. B. Harvey.

to line 10 on Sorby's scale." This includes wave lengths between approximately 0.55 to 0.44 μ .

Panceri (1878) devoted six pages of his monograph on the luminous organs of annelids to *Chaetopterus variopedatus*, describing the conditions for light production and the various regions from which light appeared. According to him the brightest areas were five in number: (1) the base of the antennae or feelers, (2) glands at the base and on the dorsal side of the wing-like processes or pinnules of the middle body region, (3) the tuberculum or suckorial disc which has the form of a small sac, (4) the edges and upper surface of three cape-like processes (paddles) of the middle region, (5) all the notopodia of the posterior region. These luminous areas are well shown in Panceri's plate reproduced in Fig. 67. He also studied the fine structure of the epithe-

lum, designating photogenic cells of two kinds, (1) spherical ones present as special masses forming the glands of the pinnules and (2) pyriform cells scattered among the ciliated epithelium of the luminous regions.

Since Panceri's time, light production in *Chaetopterus variopedatus* or *C. pergamentaceus*, which appear to be one species of worldwide distribution, has been studied in detail, by Joyeux-Laffuie (1890) in a 115-page monograph, but without too much attention paid to lumines-

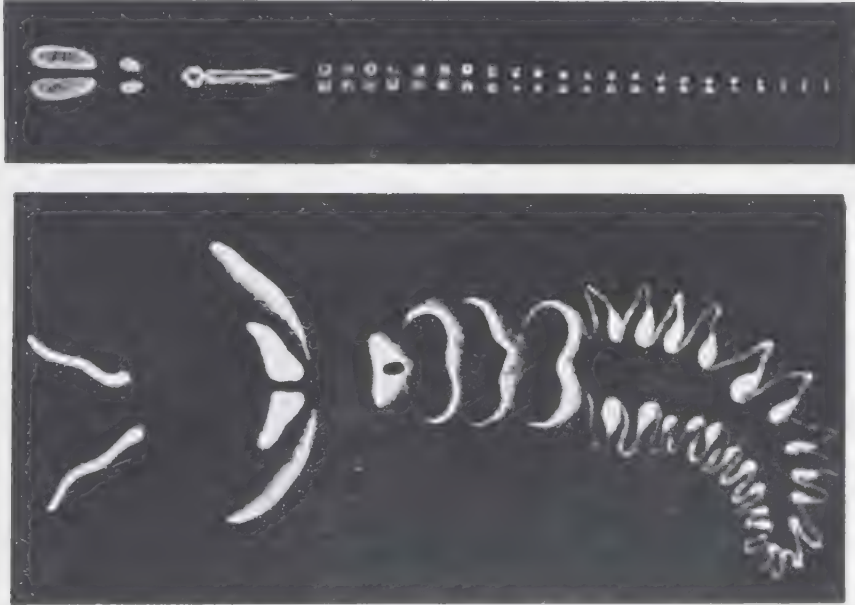


FIG. 67. Diagram of luminous areas of *Mesochaetopterus* (top), after Fujiwara and *Chaetopterus* (bottom), after Panceri.

cence, by Enders (1909), Trojan (1913), Krekel (1920), Bonhomme (1920), and Hasama (1941). Enders, who has worked out the habits and life history of *C. variopedatus*, observed that young larvae luminesce in the vicinity of the ciliated rings and older larvae in the anterior region. The eggs are not luminous. It is possible that all chaetopterids are luminous, but as yet observations are not sufficient to warrant such a statement. The regions of brightest luminescence do vary in different species. In a Japanese form, *Mesochaetopterus japonicus*, Fujiwara (1935) has described somewhat different light regions, shown in Fig. 67.

Histology. *Chaetopterus* secretes a luminous slime which comes off on the fingers when an animal is handled. The luminous epithelium is found to be a complex structure, differing in different regions, for it is also concerned with the formation and repair of the parchment tube.

which the animal never leaves. It is not surprising that some difference of opinion has developed regarding the photogenic cells. Panceri's opinion has already been given.

An early careful and detailed study of the histological characteristics of sections of the luminous regions was made by Trojan (1913) who distinguished a definite type of photogenic gland cell, filled with large granules. These were present as scattered unicellular glands among ciliated epithelial cells of the feelers, the wall of the paddles of the middle region, and the tips of posterior notopodia. In the pinnules

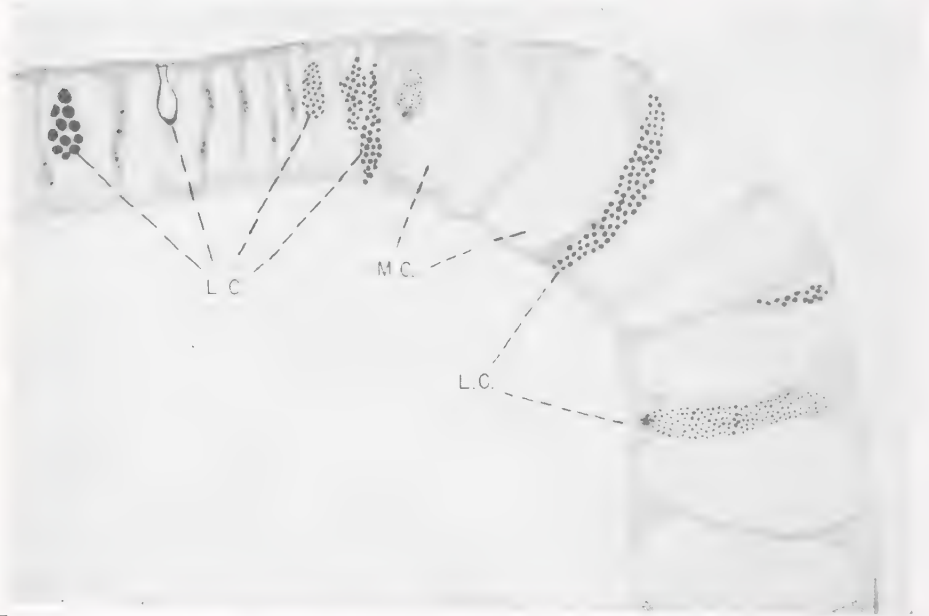


FIG. 68. Section of epithelium from edge of a fan of *Chaetopterus*, showing light cells (L.C.) and mucous cells (M.C.). After Dahlgren.

and the sac-like tuberculum the luminous gland cells lie close together and practically form a photogenic organ. Finally the basal part of posterior notopodia contains a great accumulation of photogenic gland cells which Trojan considered to be part of the end of the nephridia, practically a urinary bladder. According to Trojan, the cells which secrete the parchment-like tube are quite distinct. They contain granules and thread like inclusions, called "follicules bacillipares" by Claparède. The threads are no doubt extruded and plastered together to form the tube walls.

Dahlgren (1916) has given essentially the same account of the histology. He observed two types of cells believed to be photogenic, small oval ones, flask-shaped with ducts, embedded among the mucous cells, and elongate type extending across the epithelium and filled with fine granules, as shown in Fig. 68.

According to Krekel (1921), the luminous epithelium is made up of large columnar (rod-shaped) blue staining cells with which are often associated a small flask-shaped eosinophil variety. In the light gland of the tuberculum, Krekel noted a sudden transition between the blue columnar and the eosinophil cells, but was not certain what the relationship of the two types of cells might be.

Hasama (1941), from his study of *Chaetopterus variopedatus* common along the west coast of Korea, has distinguished among cells of the luminous regions two types, (1) cilia-free cells filled with granules, staining in toluidin blue, (2) eosinophil cells with cilia on the free surface. In the cell body there are a few toluidin blue-staining granules but relatively few compared with the first type. The more numerous the toluidin blue-staining granules, the more marked is luminescence of the region containing them.

Finally Bonhomme (1943) has made careful histological studies, using stains with an affinity for mucus. He described the luminous epithelium of the pinnules as greatly folded, forming narrow crypts where the clearest histological picture was found. The cells of this region were prismatic elements, 20 to 40 μ long, with an apical thickening of the walls which Bonhomme believed to be cytoplasmic in origin. The nuclei were squeezed against the side walls by the abundant rather large granules filling them. These granules stain in toluidin blue or polychrome blue and were regarded as mucigen granules and the cells as photocytes.

Under the microscope, with a magnification of 700 diameters, Bonhomme could see an abundance of brilliant luminous points whose light progressively disappeared. In reflected light the secretion showed granules which diminished in size, becoming less and less sharp and transforming to a homogeneous mucus layer. He therefore believed that the mucigen granules support the luminous substance making up a hydrophil complex, which becomes hydrated, swells and luminesces in contact with the water. Within the cell there is no hydration and no light emission.

The only other chaetopterid whose histology had been studied is *Mesochaetopterus japonicus*. Although the brightest luminous areas are somewhat different in distribution, the epithelial gland cells are similar to those of *Chaetopterus*. Fujiwara (1935) has observed in *Mesochaetopterus* sections, three kinds of gland cells: (1) club-shaped cells with neck and pore, scattered throughout the ciliated epithelium and containing rather small granules; (2) closely packed elongate cells along the ridge of the tentacle and in other luminous regions, filled with granules staining in toluidin blue; (3) oval cells below the surface

of the epithelium containing fibrillar contents plus very small eosinophil granules. Figure 69 is a reproduction of Fujiwara's drawing of the luminous gland cells showing four stages in formation of luminous granules.

From the various descriptions there appears to be little doubt but that the elongated columnar cells containing many granules with an affinity for mucin stains are the photogenic cells, but the relation of photogen to mucigen is not clear and must await further research on the biochemistry of light production.

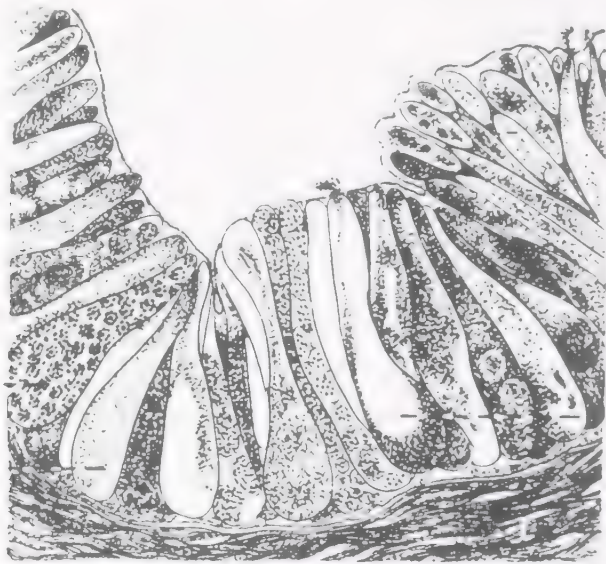


FIG. 69. Section of a luminous region of *Mesochaetopterus* showing empty cells and various stages of granule formation. After Fujiwara.

Physiology. It is probable that secretory nerves supply the gland cells and excite the secretion and that reflexes are involved in producing the light. Hasama (1941) has carried out experiments on *Chaetopterus variopedatus*, using faradic induced stimuli, which indicate that there is not only a local luminescence response in the region stimulated but that after a latent period light appears at some distance away from the stimulated spot. He found that when the duration of the stimulus reached a certain value, the duration of the luminescence was constant, lasting ten to forty seconds, independent of the time of the stimulation.

Hasama's chief interest lay in the action potential of luminescence, which he recorded with Zn-ZnSO_4 non-polarizable electrodes and a string galvanometer. With the animal in the air, one electrode was placed in the free end of the strongly luminous pinnule and the other on the weakly luminous dorsal surface of the anterior region of the

body. Electrical or mechanical stimulation of other regions then resulted in a luminescence, which after some seconds involved the pinnule. Parallel with but slightly preceding the appearance of pinnule luminescence, a monophasic potential variation occurred which rose to a maximum and then decreased, returning to the original position somewhat later than the disappearance of the light. The non-luminous region was electrically negative to luminous regions. If electrodes were placed on two luminous regions of the wing-like notopodium, no potential difference was to be observed. With chemical stimulation, the electrical variation lasted a considerably longer time than the luminescence. In addition to the luminescence potentials Hasama also observed slow rhythmic diphasic action potentials connected with waves of contraction of the smooth musculature of the worm. His description of the experiments and his records seem convincing, especially as the "luminescence potential" has been found in a number of other luminescent animals.

Biochemistry. The histochemical studies of Bonhomme (1943) indicate that the supposed photogenic granules are not lipid but mucous. No attempts to isolate the luminous substances by various chemical procedures have been attempted. Despite the brightness of the light, the author has not been able to demonstrate a luciferin-luciferase reaction. Hot water extracts of the animal which should contain luciferin, even when prepared in absence of oxygen, give no light when mixed with luminous mucus allowed to stand until the light disappears (luciferase). The *Chaetopterus* "luciferase" extract gave no light on mixing with *Cypridina* luciferin nor would *Cypridina* luciferase react with *Chaetopterus* "luciferin."

Thinking that *Chaetopterus* might behave as the fire-fly, in which McElroy (1947) has shown that addition of adenosine triphosphate to dark extracts of the lanterns will revive the luminescence, a corresponding test was made with *Chaetopterus* extracts (Harvey, 1949) but with negative results.

The luminous slime of *Chaetopterus* is somewhat more fluorescent in ultraviolet light than slime of non-luminous worms, but the difference is not striking. Whole animals exposed to sunlight will luminesce immediately on examination in a dark room. There is no marked inhibition of luminescence by light.

The luminescence of the slime is dependent on oxygen. When pure hydrogen is bubbled through a mixture of slime and sea water, the light disappears, to return again when air is admitted (Harvey, 1926).

Use of Light. It is hard to imagine what the purpose of light can be to an animal which remains hidden in a tube on the sea bottom in

mud or sand well below the surface, and which never wanders about. Dahlgren (1916) has observed eels pull *Chaetopterus* out of their tubes and has found fragments of *Chaetopterus* in their stomachs. A predacious eel is illustrated in Fig. 70, and it may be supposed that



FIG. 70. *Chaetopterus* in its tube, attacked by an eel. After Dahlgren, from a drawing of Bruce Horsefall.

the eel or some other animal is not always so successful in attack but is scared away by the luminescence, which may serve as a warning signal.

Cirratulidae

In making a systematic study of alleged luminous algae Molisch (1904) discovered at Trieste a small cirratulid worm, *Heterocirrus saxicola*, responsible for the light but made no detailed observations on the light production. Lund (1911) also has reported that during the summer in the harbor of Montego Bay, Jamaica, cirratulids liberate a yellowish luminescent secretion.

The only detailed study of luminescence in this family has been made by Bonhomme (1944) on *Heterocirrus bioculatus*, living on algae of salt water canals near Sete on the Mediterranean. In June the worms are just beginning to luminesce, but during summer months the intensity increases and adult individuals are abundant in July and August when they appear like a veritable shower of yellow green luminous particles. *Heterocirrus* does not readily respond to chemical

and tactile stimuli, nor luminesce when immersed in a fixative but regularly emits light in fresh water.

At first Bonhomme was inclined to believe the setae were luminous but discovered that this appearance was due to a luminous secretion adhering to them. The secretion comes from paired lateral regions in the posterior part of the worm. Examined in water under a binocular microscope, the luminous plates were seen to be at first composed of a large number of separate luminescent points. After this first emission, the entire abdominal integument appeared diffusely luminous, and the light slowly disappeared.

Histology. Sections of the integument made from animals caught in June show a few large mucous cells scattered in an epithelium of pigmented supporting cells, immediately over muscular layers. Externally there is a rather thick cuticle. Sections made from animals caught in August are found to be almost entirely made up of the large egg-shaped mucous cells, with only a few supporting cells between them. The large mucous cells are undoubtedly the "photocytes." They probably open through pores in the integument although these are difficult to see. Bonhomme considered that the mucus contained the luciferine-luciferase combination, which emits light in the cell at the moment of hydration of a "mucigene," destined to form the mucus. The luminous mucus then diffuses slowly out of the cell, in contrast with the condition in *Chaetopterus*, where abundant mucus secretion is formed.

The presence of luciferin and luciferase has never been tested in this animal nor is anything known of the biochemistry of light production.

Terebellidae

In recent times a number of workers have described the light of terebellids, among them Grube (1861), who noticed their violet light during a trip to Trieste. *Polycirrus aurantiacus* and *P. medusa* occur there and also at Naples where they were studied by Panceri (1878). *Polycirrus medusa* differs from *P. aurantiacus* in that only the body is luminous and not the tentacles. In *P. aurantiacus* the long tentacles (cirri) and the body emit a light, also described as violet by Panceri, each tentacle individually on stimulation, so that the appearance is that of a colony of luminous worms. Placed in fresh water the light becomes continuous, gradually going out in the course of fifteen minutes.

Dahlgren (1916) studied various species of *Polycirrus*, and the author has observed *Polycirrus phosphoreus* living on oysters along the Jersey coast and also the English species, *Polycirrus caliendrum*, from Plymouth. Fig. 71 shows the general form of the worms.

The snake-like tentacles of *Polycirrus aurantiacus*, observed through the microscope, are a striking sight. The walls of the tentacle with its ciliated feeding groove are yellow, contrasting with the red blood within. Each red blood corpuscle can be clearly seen as it is forced along the thin-walled vessel in the tentacle, which serves as a filamentous gill.

Histology. The muscles of the tentacle and the unicellular glands (folliculi) with a secretory pore, scattered irregularly in the epithelium, can be clearly seen in the living animal. Panceri noticed yellow refringent material within the folliculi and designated them photogenic cells, as they resembled similar ones in *Chaetopterus* and *Balan-*

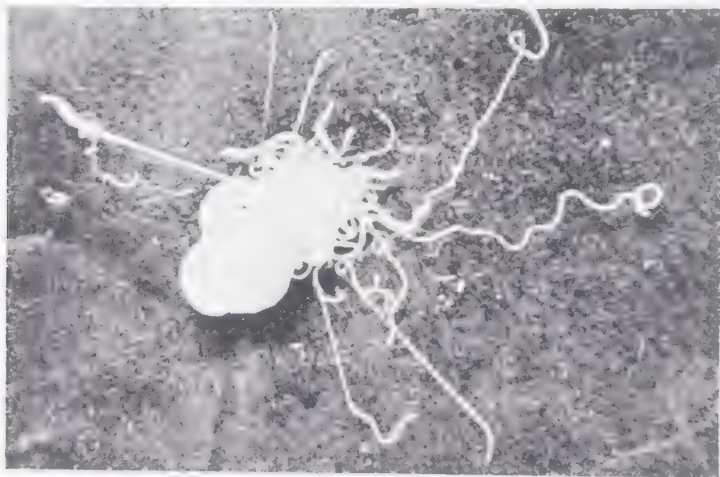


FIG. 71. *Polycirrus phosphoreus*. Photo by E. B. Harvey.

glossus and were lacking in the tentacles of *P. medusa*, which do not luminesce.

Sections of the tentacles of *P. aurantiacus* have been described by Dahlgren (1916), who was able to distinguish clearly the cells containing large masses of yellow material, which give the yellow color to the tentacle and are also believed to form a substance distasteful to other animals. Eight other types of cells, some of them representing stages of development, were described in the epithelium, and it is obviously difficult to pick out those producing the luminous secretion. Dahlgren called the presumed photocytes "black-capped cells," but there is no actual proof that they produce the external luminous secretion.

Another study has been made by Tanassicūk (1926) of *Polycirrus albicans*, obtained at the Biological Station at Murmansk, Russia. In this species the tentacular and body epithelium has been entirely transformed into a glandular coating. Curiously enough, although Tan-

assiicük does not mention Dahlgren's study, he also lists eight kinds of unicellular glands, some of which may be stages in development. Four of these stain in Heidenhain's hematoxylin and the other four do not. Some cells contain fine granules and others rods. Those with granules are believed to be the photogenic ones, at least they are similar to the luminous gland cells of *Chaetopterus*.

Biochemistry. Concerning the luminous material, practically nothing is known. The author (1926) studied *Polycirrus caliendrum* at Plymouth, England, but was unable to demonstrate a luciferin-luciferase reaction. Cold water extracts of the worm which should have contained luciferase did not luminesce on mixing with *Cypridina* luciferin nor did hot water extracts of *Polycirrus* light the *Cypridina* luciferase.

The effect of oxygen lack on *P. caliendrum* was not tested but oxygen is necessary for luminescence of another species. The author has placed whole animals of an allied form, *Thelepus cincinnatus*, found at Naples, in a tube together with the luminescent slime and has bubbled hydrogen through the tube. After a time the light disappeared, to return on admitting air. The slime of *Thelepus cincinnatus* shows no special fluorescence in ultraviolet light (without the visible).

OLIGOCHAETA

General

That earthworms can luminesce has been known since 1670, when Grimm described scarlet worms, probably earthworms, from the Coromandel coast of India. The next record is more than a century later. Flaugergues in 1780 wrote a letter to M. le Baron de Sevières, published in the *Journal de physique*, giving an account of specimens observed along the Rhone. He described the whole body as like rotten wood but more luminous on the clitellum and noted that no light came from a dead worm.

A little later, in 1793, Brugière described the light from earthworms at Avignon and, beginning around 1840, many accounts have appeared from time to time reporting luminous earthworms in this or that locality. Among these authors were Duges (1837), Evermann (1838 in Russian), Forester (1840), Audouin (1840), Allman (1843), Cox (1853), Cohn (1873), Panceri (1875), von Stein (1883), Vejdowsky (1884), Atkinson (1887), Hakker (1887), Kolbe (1887), Giard (1887), Monier (1889), Barrois (1891), Matzdorff[†] (1893), Friend

[†]This paper discusses identification of the species described in earlier records of earthworm luminescence.

(1893-1924), Lloyd Bozward (1897), Beddard (1899), Walter (1909) and Ssuchoterin (1910), both in Russian, Issatschenko (1911) in Russian with German summary, Linsbauer (1910, 17), McDermott and Barber (1914), Stephenson (1914, 30), Dubois (1914), Gates (1925, 44), Pickford (1937), Haneda and Kumagai (1939) in Japanese, Bahl (1943), and Ray (1945). The accounts frequently express astonishment that earthworms should luminesce. They have a parallel in a similar series of reports on the luminescence of myriapods, animals living under somewhat the same general conditions as earthworms.

Earthworms are worldwide in distribution and the list of reported luminous forms includes some twelve genera, of five different subfamilies. The luminous genera (in italics) of oligochaete families listed earlier, are given in the following table, kindly revised for me by Dr. Grace E. Pickford. Families of oligochaetes containing luminous genera are in italics.

- Enchytraeidae* (21 genera including ?*Enchytraeus*, ?*Michaelseniella*, *Henlea*)
Megascolecidae
 Acanthodrilinae (16 genera, including *Eodrilus*, *Microscolex*, *Parachilota*)
 Megascolecinae (24 genera, including *Lampito*, *Pontodrilus*)
 Octochaetinae (23 genera, including *Eutyphoeus*, *Ramiella*, *Octochaetus*)
 Ocnodrilinae (14 genera)
Lumbricidae (8 genera, including *Eisenia*)

Region of Luminescence

All observers agree that earthworms produce a luminous slime, which comes off on the fingers, but there has been some dissent on the origin of the luminous material. Most early authors called the whole external surface luminous. Panceri (1875) described secretion from special glands on the girdle (clitellum), and many subsequent writers like Giard (1887) have spoken of epidermal glands, but this view is incorrect.

That the luminous material comes from the coelomic fluid rather than epidermal glands, at least in some species, was first pointed out by Benham (1899) of Dunedin, New Zealand, who wrote:

"Our large white earthworm (*Octochaetus multiporus*) has a milk coloured coelomic fluid of very great tenacity; it can be drawn out into strands, and soon hardens on exposure to air. In the dark, when the worm is handled, this fluid is discharged abundantly from the dorsal pores and from the mouth, which it reaches through the protonephridia opening into the buccal cavity. The fluid is brilliantly phosphorescent when freshly discharged, and the fluid sticks to one's fingers very persistently; but it soon loses its phosphorescence. I wish here merely

to point out that the luminosity is due to the coelomic fluid in *O. multiporus*, and I believe that further examination will show that the same is true of *Allobophora foetida*.

"The fluid in *O. multiporus* contains numbers of 'elaeocytes,' which are present also in *A. foetida* and other European worms; but in the New Zealand worm they are colourless, not yellow. A very remarkable kind of corpuscle is also present, viz., a cell containing a thread-like structure not unlike those described by Goodrich in an enchytraeid a few years back. I am now endeavouring to locate the phosphorescence—that is, to ascertain which of these two cells is the seat of the phenomenon."

Somewhat later Gilchrist (1919) made a most valuable and extensive study of a South African form, *Chilota* sp. found on Table Mountain.* These worms were not luminous when first dug up, but remained rather rigid, only later becoming motile and discharging the luminous mucus from mouth and anus, chiefly the former. By flexing the body the liquid could be thrown a considerable distance. The fluid actually came from the coelom, passing into the mouth and anus by pores. In the fluid, whose light may last over an hour, were to be found cells containing greenish granules. On adding water to the cells the granules dissolve and emit light. Gilchrist obtained a number of photographs of the light showing the outline of the animal by pressing the worm on a photographic plate in the dark and then developing the plate.

The observations of Gates (1925) on Burmese earthworms are in agreement with those of Benham and Gilchrist. Of seventeen species of earthworms obtained in Rangoon, four turned out to be luminous to different degrees. These were *Eutyphoeus peguanus*, *E. foveatus*, *E. rarus*, and *Megascolex* (*Lampito*) *mauriti*. They were never observed to luminesce without rather violent stimulation, even when large numbers were together in a glass jar for long periods of time. Gates wrote: "Allowing the worms to crawl on dry and wet, rough or smooth surfaces has failed to result in the production of light. Rubbing gently and shaking violently in water have also produced no positive results. . . . The most satisfactory method of demonstrating the photogenic ability of the worm is to drop the animal into a finger bowl containing a very weak solution of ammonium hydroxide in water. The worm lies quietly for a moment and then commences

*According to Pickford (1937) the worm studied by Gilchrist was either *Parachilota olgoensis* or *P. bergvleitani*. The latter is brightly luminous as are a number of species of *Eodrilus*, observed by Pickford to discharge the luminous fluid from mouth and anus, but only after considerable irritation.

to writhe around in a rather deliberate fashion. After a varied amount of this writhing, mucus is suddenly shot out from the dorsal pores along more or less the whole length of the body. The light is not produced immediately on the discharge of the mucus but appears only after the lapse of a short interval, the light gradually increasing until it reaches a maximum amount."

Gates (1944) has recently discovered that the coelomic fluid of the three species of *Eutyphoeus*, *E. incommodus*, *E. nicolsoni*, and *E. waltoni*, found at Allahabad, India, also luminesces in ammonia solution, and he is inclined to believe that all species of this genus are luminous. Specimens of *Ramiella nainiana* were also luminous but not *R. cultrifera*, and *Octochoetoides fermori* was non-luminous also. Bahl (1943) has likewise recorded the luminescence of *Eutyphoeus*, common at Lucknow during the rainy season, and told me that he regularly made a classroom demonstration of the luminous mucus in ammonia solutions.

A rather unusual sea earthworm (*Pontodrilus matsushimensis*) was discovered in Japan by Kanda and Haneda in October, 1936, living in the wet sand at the tidal line near Yokohama. It is widely distributed in tropical countries of Asia. A yellow luminous fluid containing no luminous bacteria is secreted from mouth and anus on strong irritation or injury.

Use of Light

It was suggested by Flaugergues in 1771 that the light disappeared after copulation and might be involved in sex attraction, but Friend (1919) has pointed out that the luminous earthworms in England have no eyes or rudimentary ones, while Gilchrist (1919) believed the purpose of the light was to scare predacious animals by throwing slime from the burrow or by leaving a mass of luminescent slime behind as they moved away. The luminescence seems to appear chiefly when the animal is severely irritated.

Physiology

Very little is known of the mechanisms involved in the ejaculation of coelomic fluid. Most observers describe the luminescence of earthworms as difficult to excite, although such is not always the case. Both Lloyd-Bozward (1897) and Beddard (1899) say that the slightest irritation, such as stamping on the lawn, will cause the English earthworm (probably a *Microscolex*) to light up. Once this sensory reflex is started, the effectors are no doubt muscles which force the coelomic fluid to the outside. There is possibly a seasonal variation,

perhaps connected with a sexual cycle, when the reflex is more easily elicited.

Skowron (1926) noticed that a day night rhythm of luminescence existed in *Microcolex*, studied at Naples, in that the discharge of luminous material occurred more easily at night than in the daytime. Once discharged, however, there was no inhibition of the luminescence itself by daylight or artificial light. Later studies of Skowron (1928) on specimens of *Microcolex phosphoreus* obtained in the coal mines near Cracow indicate a different behavior. "Visiting this place I observed in one passage not used for over two years about 230 meters below the surface, great quantities of *Microcolex* which in these special conditions of constant temperature and moisture had propagated very rapidly. Walking in darkness, hundreds of luminous points were seen, glowing brilliantly after every step. . . .

"Contrary to the individuals I have examined in Naples and others which were recently sent to me from Naples, the coal-mine forms do not show the day-night rhythm in luminescence, a fact probably connected with the constant darkness in which they are living. The degree of irritability of the nervous system, regulating the ejaculation of the luminous slime, seems to be the same during the night and day time, owing to these exceptional conditions."

Relation to Luminous Bacteria

A number of writers including Haupt (1903) have suggested that earthworm luminescence might be due to luminous bacteria. Issatschenko (1911), who isolated *Photobacterium chironomi* from luminous midges, attempted to obtain bacteria from the earthworm, *Henlea ventriculosa*, sent him by Walter, but without success. Molisch (1904), Dubois (1914), and Linsbauer (1917) were also unable to demonstrate luminous microorganisms. Dahlgren (1916) had suggested that since earthworms eat vegetable matter, the luminescence might be due to fungi but without attempting an experimental verification.

In 1922 Pierantoni revived the bacterial theory of origin of earthworm light as a result of his study of the form common in Europe, *Microcolex phosphoreus*. He described granules and rods in the cells of the coelom, which he believed to be luminous bacteria and to pass through connective tissue and two layers of muscles to reach the skin glands. He also found these bacteria in the non-luminous genus, *Pheretina*. Growth of the bacteria on culture media never resulted in luminous colonies.

Exception has been taken to Pierantoni's views by Knop (1926) and

Skowron (1926, 28), who both worked on *Microscolex phosphoreus*. Skowron observed that the yellowish green luminescent slime came, not from skin glands or skin excretory pores but from anus and mouth, usually the former. Under the microscope Skowron could easily observe in the slime rounded coelomic cells containing strongly light refracting granules in their protoplasm and scattered through the fluid. The granules are the source of the luminescence, but their behavior on adding various chemicals or under various conditions differed radically from the behavior of luminous bacteria. For example, small amounts of Na fluoride added to luminous bacteria quench the light immediately, whereas fluoride has no inhibiting effect on the slime of *microscolex*. Komarek (1934), also, was unable to find bacteria responsible for the light of a common earthworm, *Eisenia submontana*, living under the bark of trees in the forests of Carpathian Ruthenia. So many facts have been presented against the bacterial theory of their luminescence that there can be no doubt of the self-luminosity of earthworms.

Biochemistry

All observers have attributed the luminescence of the excreted coelomic fluid to the granules in its cells. This fluid continues to glow for a half hour if kept moist. After drying, addition of water will again result in luminescence (Gilchrist, 1919; Pickford, 1937). Whole worms also can be dried and kept in a desiccator for at least three months and will luminesce when moistened (Skowron, 1926).

A careful study of the excretion has been made by both Gilchrist (1919) and Skowron (1926) who are in agreement on the general behavior. The cells of the coelomic fluid are rather large, up to 20 μ in diameter, and are packed with granules of various sizes, of a faint greenish color, highly refractive and without optical activity. The cells disintegrate, liberating their contents, but light still appears in a filtrate of the liquid through filter paper, which allows granules to pass. Gilchrist found that light from the granules could be detected if a thin film of the liquid was allowed to dry on a microscope slide and then moistened, "when an area of bright luminous specks, like a starry sky, passed slowly over the field of vision, at that point of contact of the water and the dried substance. . . . The luminous particles were found amongst the discharged granules of the cells in abundance."

According to Skowron, under high magnification the granules can be seen to be composed of two substances, one of which forms the central part while the second lies around the periphery. The granules differ from coelenterate or ctenophore granules in resistance to cytolytic

agents like chloroform or saponin and to osmotic pressure changes or various salts, which do not affect their solution to any great extent. For example the slime glows as brightly, and the granules dissolve as rapidly in salt solutions as in distilled water. In high concentrations of glucose and glycerine the light disappears but returns on diluting with water. Ether is the one cytolytic agent that readily evokes luminescence.

It is attractive to assume that the two substances visible in the granule are luciferin and luciferase and that dissolution of a film separating them allows mixture and results in luminescence. Gilchrist (1919) has been unable to demonstrate luciferin and luciferase in *Chilota* sp., and the author (1926) obtained negative results with *Microcolex phosphoreus* even when the luciferin solution was prepared in absence of oxygen. A cold water extract of the slime which should contain luciferase gave no light with *Cypridina* luciferin, and a hot water extract of *Microcolex*, which should contain luciferin, also remained dark on mixing with *Cypridina* luciferase. Of course, there may be other substances necessary for luminescence which are lacking in luciferin or luciferase solutions prepared in the classical manner. The role played by adenosine triphosphate has not been investigated and leaves a definite gap in our knowledge.

The necessity of oxygen for luminescence of earthworms has been established by Gilchrist (1919), Harvey (1926), and Skowron (1926).

When *Microcolex phosphoreus* is examined in near ultraviolet light (without the visible), yellowish fluorescent areas can be observed over the external surface in no regular position. As other non-luminous species also show this yellow fluorescence, the author (1926) concluded that it was only fluorescence of the slime so common on all earthworms.

Such an interpretation is probably incorrect. That fluorescent materials may be involved in earthworm luminescence is indicated by the studies of Komarek and Wenig (1938) and Backovsky, Komarek, and Wenig (1939). The papers are in Czech, so that only the English summary has been available. There are two species of *Eisenia* which occur in Carpathia, *Eisenia submontana*, described by Vejdowsky in 1875, a luminous form, and *E. foetida* which is non-luminous. These earthworms furnish perfect material for the important comparison of a luminous and non-luminous animal.

The bioluminescence, yellow green in color, comes from the yellow colored granules of the coelomic lymphocytes, which are very numerous. If these lymphocytes are heated to abolish their bioluminescence and then illuminated by ultraviolet light, the heated lymphocytes fluoresce yellow green. However, if the lymph is allowed to stand

until its bioluminescence has disappeared and then tested with ultraviolet, the fluorescence is blue.

In the non luminous form, *E. foetida*, the lymph fluoresces in ultraviolet light with a yellow-green color, like the bioluminescence of *E. submontana*, and does not change on standing. If irradiated for a considerable time with ultraviolet light, however, the yellow-green fluorescence does change to a blue fluorescence. The yellow-green fluorescence is believed by the Czech authors to be due to riboflavin and the blue fluorescence after bioluminescence has ceased, or after treatment with ultraviolet light, to lumiflavin. These authors give chemical and spectroscopical evidence to indicate that riboflavin is present in the lymphocytes of both luminous and non-luminous earthworms, but that in the luminous form it is changed to lumiflavin (with blue fluorescence) during bioluminescence. Since light also converts lactoflavin to lumiflavin, it was suggested that "special chemical processes in the luminous yellow granules [of *E. submontana*] are accompanied by the production of short waved rays" and that "the light produced during the decomposition of the lymphocytes of *E. submontana* is the fluorescence." The difference between the luminous and the non-luminous earthworm therefore lies in the lack of factors converting lactoflavin to lumiflavin in the non-luminous species.

In a later very extensive paper in Czech, Wenig (1946) has given additional evidence for the existence of lactoflavin in the granules of the two species of *Eisenia*. The fluorescence maximum is at 5,500 Å and fluorescence intensity depends on the pH. It disappears at pH < 1 and at pH = 12 shifts to the blue. From the curve relating fluorescence intensity to pH it was clear that the undissociated molecules or amphions are the source of the light. The acid dissociation constant K_a is 59.5×10^{-12} ($pK_a = 10.23$) and the basic $K_b = 0.41 \times 10^{-12}$ ($pK_b = 1.61$), agreeing with the values for lactoflavin. In addition there was present in alkaline solution a blue fluorescent material of unknown nature.

The change in the earthworm pigment under the influence of ultraviolet light in neutral and weakly acid media is also similar to change accompanying photolysis of lactoflavin to lumichrome, and in alkaline solution a chloroform soluble pigment, identical with lumilactoflavin, appears. Moreover, the reduction of the granule pigment by tin in strong acid solution gives the red semiquinone of lactoflavin, and the reduction potentials as well as the dissociation constants of the reduced form ($pK_{LA} = 6.3$) and the oxidized form ($pK_{\infty} = 10.2$) agree with lactoflavin. There can be no doubt of the presence of lactoflavin in

* Komarek and Wenig (1938) mentioned the possibility of mitogenetic rays.

these granules of the earthworm lymphocytes. Lactoflavin has been found in the lanterns of the fire-fly (Brooks, 1940, and Ball and Ramsdell, 1944).

In a recent note, Wenig and Kubista (1949), using the Crammer partition paper chromatography method, have determined that riboflavin itself and not flavin phosphate or flavin adenine dinucleotide is present in both luminous *E. submontana* and non-luminous *E. foetida*. The difference in luminosity must therefore be sought in some other factor than the type of flavin present.

The relation to oxygen and the presence of luciferin and luciferase were tested by Komarek and Wenig (1938), whose conclusions are summarized by Wenig (1946, p. 355) as follows:

"The bioluminescence of the earthworm *Eisenia submontana* takes place only in liquid media in the presence of molecular oxygen. Oxygen cannot be replaced by the other oxidising agents except hydrogen peroxide. Cyanides do not inhibit the luminous reaction.

"The luminous capacity of the extracts cannot be regenerated either by reduction on the electrode, or by hydrogen in *statu nascendi*, prepared with pulverised Zn or Mg, or by reduced dyes.

"Bioluminescence disappears under the influence of an elevated temperature. In the extracts a thermolabile and thermostabile component of the luminescent system can be differentiated in analogy to the luciferase and luciferin of other authors. The possibility of the thermolabile component being an enzyme with such properties as those supposed in luciferase is discussed."

The mechanism of luminescence is also described by Wenig: "Molecules of lactoflavin, adsorbed in an oriented layer on the surface of granula of lipoid character, are considered as the source of light. The activation energy which brings them into an excited state is probably derived from an oxidative reaction in which molecular oxygen takes part. The possibility is discussed whether the energy set free by the oxidation of dihydroflavin is sufficient for the activation of the neighbouring molecules of flavin, enabling them to radiate light of the corresponding colour." Since the energy discussion is in Czech, the author was unable to follow it.

CHAPTER VIII

Mollusca Except Cephalopoda

CLASSIFICATION

The great group of molluscs has been divided by J. Thiele into six classes with groups containing luminous species in italics, as follows:

Aplacophora¹ or Solenogastres (5 families, including *Chaetoderma*)

Polyplacophora or Loricata (Chitons 2 orders, 6 families)

Gastropoda (Periwinkles, snails, conches, cowries, etc.)

Opisthobranchiata (sea hares, pteropods and nudibranchs)

 ?*Prosobranchiata* (sea snails, limpets, abalones, etc.)

Pulmonata (fresh water and land snails and slugs)

Scaphopoda (Tooth shells, Dentalidae and Siphonodentalidae)

Pelecypoda or *Bivalvia* (Bivalves)

 Anisomyaria

 Taxodonta

Eulamellibranchiata

 Anomalodesmata

Cephalopoda (Squid and octopi)

 Tetrabranchiata (*Nautilus*)

Dibranchiata

Vampyromorpha (*Vampyroteuthis*)

Decapoda (Squid and *Spirula*)

 ?Octapoda (Octopus, devil fish, and argonaut)

Of these classes, luminescence is widespread among the *Cephalopoda*, where complicated luminous organs or photophores have been developed, rare among the *Pelecypoda* and *Gastropoda*, and absent in the *Scaphopoda*, *Solenogastres* and *Loricata*. Six genera of *Gastropoda*, *Phillirrhoë*, *Tethys*, *Kaloplocamus*, *Plocamopherus*, *Latia*, and *Dyakia*, are known to be luminescent, and *Tonna* has been reported luminous. Three genera of *Pelecypoda*, *Pholas*, *Barnea*, and *Rocellaria*, boring bivalves of the *Eulamellibranchiata*, are also of undoubted luminosity.

Species of doubtful luminescence are found among the pteropods and

¹ Sometimes considered a separate phylum. The *Solenogastres* and *Loricata* are sometimes combined as *Amphineura*.

a few others such as *Aeolis* (now *Aeolidia*) and *Glaucus*: the heteropod, *Pterotrachea* (or "Nucleus"); the land snail, *Helix*. They will be considered in their proper group.

GASTROPODA OPISTHOBRANCHIATA

The relation of the pteropods and the nudibranchs to the rather large number of families of Gastropoda Opisthobranchiata is brought out in the following classification of J. Thiele. All luminous genera are in italics.

Gastropoda Opisthobranchiata

Pleurocoela (Pteropods and sea hares)

Cephalaspidea (12 families, 33 genera)

Pteropoda thecosomata

Spiratellidae (*Spiratella*, Thielea)

?*Cavolinidae* (?*Creseis*, ?*Styliola*, *Hyalocyclis*, ?*Clio*, ?*Cavolinia*, Cuvierina, Diacria)

Peraclidae (*Peracle*)

Procymbuliidae (*Procymbulia*)

Cymbuliidae (3 genera)

Desmopteridae (*Desmopterus*)

Anaspidea

Aplysiidae (8 genera)

Pteropoda gymnosomata (6 families, 13 genera)

Saccoglossa (5 families, 12 genera)

Acoela (Nudibranchs)

Notaspidea (3 families, 11 genera)

Nudibranchia

Doridoxidae (*Doridoxa*)

Bathydorididae (*Bathydoris*)

Hexabbranchidae (*Hexabbranchus*)

Dorididae (*Chromodoris* and many others)

Ancylodoridae (*Ancylodoris*)

Polyceridae or *Euphuridae* (*Triopa* or *Euphurus*, *Plocamopherus*, *Kaloplocamus* and 31 others)

Doriopsidae (*Doriopsis*, *Doriopsilla*)

Phyllidiidae (5 genera)

Hypobranchiaeidae (*Hypobranchiaea*)

Goniaeolididae (*Goniaeolis*)

Duvauceliidae (8 genera)

Doridoididae (*Doridoides*)

Arminidae (*Pleuroleura*, *Armina*)

Hedylidae (*Hedyle*)

Dironidae (*Dirona*)

Dendronotidae (*Dendronotus*, *Campaspe*)

Scyllaeidae (*Scyllaea*, *Crosslandia*)

Bornellidae (*Bornella*)

Tethyidae (*Tethys*, *Melibe*)

Lomanotidae (Lomanotus, Hancockia)
Phyllirhoidae (*Phyllirhoë*, Ctilopsis, Cephalopyge)
 Proctonotidae (Proctonotus, Janolus)
 Notaeolidiidae (3 genera)
 ?*Aeolidiidae* (12 genera, including ?*Aeolidia*)
 ?*Glaucidae* (?*Glaucus*)
 Fionidae (Fiona)
 Heroidae (Hero)
 Induliidae (Indulia, Caecinella)
 Myrrhinidae (Myrrhine)
 Calmidae (Calma)
 Rhodopidae (Rhodope)

Pteropods

The number of pteropods which are said to be luminous is small and the observations are old. These include *Cleodora*, now *Clio cuspidata* (Bennett, 1837), *Creseis conica* (Baird, 1831), *Hyalea* (now *Cavolina*), and *Styliola*, all mentioned by Giglioli (1870), Della Valle (1875, p. 43) and Gadeau de Kerville (1890). These four genera all belong in the *Cavolinidae* of the *Pteropoda thecosomata*.

There is every reason to expect the development of luminescence among the pteropods since they are transparent marine plankton forms, among which luminescence is a common phenomenon. However, there have been no modern studies or even reports of luminous pteropods, and the older records must be considered somewhat doubtful. Probably the most trustworthy is that of Bennett (1837), writing "On marine Noctilucae," who said, "An interesting fact noted by the author is that the *Cleodora cuspidata*, which is found floating in great numbers on the surface of the sea in various parts of the Pacific Ocean, exhibits a speck of delicate blue light shining through the apex of its extremely thin shell."

The Baird (1831) reference is less convincing. In an article "On the Luminousness of the Sea," Baird referred to the work of Macartney (1810) and gave figures of a number of luminous animals, but it is not clear whether he saw all these forms actually luminesce. One figure shows a transparent specimen in a conical shell, clearly a pteropod, called *Chryseis conica*, but there is no statement that it is luminous, whereas of a *Salpa*, Baird says, "It retained its luminous property for upwards of 12 hours after it was put in a tumbler of clear salt water."

Nudibranchs

The nudibranchs or sea slugs are a group of gastropods in which the shell is absent but whose mollusc characteristics are unmistakable. Frequently they are brilliantly colored and possess rows of branching

respiratory tufts. Reports of luminosity in this group appeared fairly early in the last century. The Reverend Wm. Kirby, co-author with Wm. Spence of the famous "Introduction to Entomology" (1817), wrote another book in 1835, *The Creation of Animals and Their History, Habits and Instincts*. In this book Kirby mentioned violet snails (*Lanthina* of Bosc) which float at the surface of the sea and color the water with a blue fluid when disturbed and "are vividly phosphoric at night." Just what these snails were cannot be determined, but they were probably nudibranchs.

Another observation was made by Lowe in 1842 which definitely established what he called *Peplidia* (now *Plocamopherus*) as luminous. Since then Elliot (1908), Risbec (1925, 28), under the name *Triopa* or *Euphumis*, Baba (1935), Okada and Baba (1938), and Hasama (1943) have studied this genus, and Kato (1949) has found a luminous allied genus, *Kaloplocamus* (or *Caloplocamus*). The best known of these molluscs is the transparent pelagic *Phyllirrhoë*, first carefully studied by Panceri (1873) whose work is classical.

In addition to the families containing these genera (*Polyceridae* and *Phyllirrhoidea*), three other families contain reported luminous forms, the *Tethyidae*, *Aeolidiidae*, and the *Glaucidae*. *Glaucus*, according to Mangold (1910, p. 274) was seen to luminesce by Vayssière, but no reference is given. A young luminous *Aeolis* (now *Aeolidia*) was mentioned by Gadeau de Kerville (1890, p. 145), but again no reference is given and the author has been unable to verify the report.

Concerning luminescence among sea hares nothing can be said except to refer to the original reference. When Grube (1861) made his collecting trip to the region of Trieste, he observed a number of luminous animals, among them a *Tethys*² fimbria, brought to him by fishermen of the island of Lussin along the Dalmatian coast. He kept it in a bucket of water and noted that "it phosphoresced strongly in the dark if I disturbed it or if I moved my hand in the water. Less strong were the light points if it was left alone." He published an excellent colored plate of the animal.

Plocamopherus. The nudibranchs of the genus *Plocamopherus*, family *Polyceridae* or *Euphumidae*, were first recorded as luminous by Lowe (1842), who called one *Peplidia Maderae* and studied its movements and luminescence for several days. Lowe wrote, "At night, especially when thus in motion, it appeared most brilliantly phosphorescent; the light flashing progressively but very rapidly along the body."

² The similar name *Tethys* has been applied to a species of *Salpa*, but the specimen of Grube was a nudibranch, as is apparent from his figure.

³ Sometimes spelled *Plocamophorus*.

especially from all the branchial tufts and the edges of the veil and crest."

In 1908, Elliot described an allied species, *Plocamopherus ocellatus*, living at 5 fathoms in the mud of Suez Bay and in boxes where oysters are kept. In daytime the animals were sluggish, but at night they actively crawled and even swam. "When undisturbed, they often emit a fairly bright light which glows steadily for about 5 seconds, goes out, and reappears after an interval of 5-10 seconds. This is emitted from the tip of one of the pairs of dorsal processes situated halfway between the head and the gills. . . . Occasionally bright flashes may be given off by the gills. . . .

"On touching the surface of the water or flicking it with one's finger, a brilliant blue-green flash of light comes from the gills of each specimen. The gill is shown up plainly and shown to be half-contracted. The light comes from the finer branches, and the larger ones show dark against the light. On flicking the water, the light ceases to be shown after a second or third time. If now any part of the body is touched, a brilliant series of rapid flashes lasting up to 5 seconds appears from the gills, the effect being like miniature tropical lightning. This also ceases after the third or fourth irritation. The animal may take to swimming, and then clouds of mucus fill the water with light.

"The strong irritation of a fresh specimen results not only in the main display from the gills, but also a milder yet brilliant and steady light is emitted from the edges of the tail, dorsal crest, and various points and processes of the body."

The next record of a luminous nudibranch comes from Risbec (1925), an animal named provisionally *Triopa* (*Euphonia*)⁴ *fulgurans*, about 15 mm long from Noumea Bay, New Caledonia. Risbec described the brief flashes of light given off by the animal when disturbed and believed that the "luminous emission is without doubt a means of defence." In a later paper Risbec (1928) gave the animal a new generic name, *Plocamopherus fulgurans*. Another luminous species, *P. ceylonicus* Kelaart, was also described, about 25 mm long, abundant on the Coromandel coast of India. Its luminescence is brighter and a little yellow and its flashes less rapid. Excellent colored plates of both species accompany the article.

Okada and Baba (1934) mention four species of *Plocamophorus* as luminous, *P. maderae* (Lowe, 1842), *P. ocellatus* (Elliot, 1908), *P. fulgurans* (Risbec, 1925), *P. ceyloni* (Risbec, 1928), and add a fifth, *P. tilesii* Bergh from Japan, observed by Baba (1935) in Mutsu Bay. A beautiful colored plate of *P. tilesii*, taken from the paper of Baba, is

⁴ Probably *Euphumus*.

reproduced as Fig. 72. The dorso-lateral processes of *P. tilesii* are especially luminous and have "light sacs" as well as muscle bands to force out the luminous secretion.

Histology. Okada and Baba (1934) have made careful studies of the histology of this form whose epithelium contains photogenic, mucous, and albuminous cells. The tall columnar "luminous" cells are clear and colorless, with a fine granular cytoplasm, and stain with eosin. They are found over the whole body but are most marked on dermal papillae. The mucous cells are of the typical goblet type staining in hemotoxylin. There are also present eosinophil albuminoid

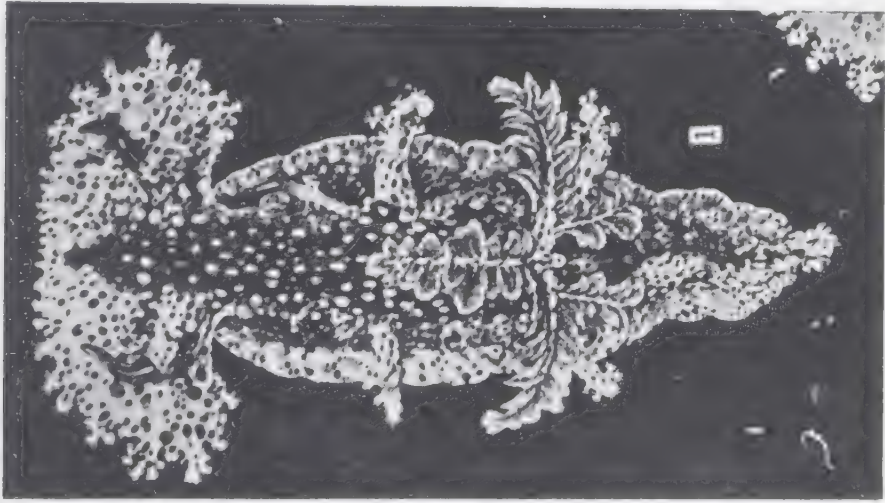


FIG. 72. *Plocamopherus tilesii*. After Baba.

cells, with coarse granular cytoplasm, "in the fresh state tinged with yellow." In addition, Okada and Baba refer to sacs containing rods like bacteria, which are found under the epithelium.

Hasama (1943) has also studied the histology of *Plocamopherus tilesii*, finding the same types of cells as Okada and Baba. He described many fine granular eosinophil cells, together with some mucous cells as forming clumps on the dorso-lateral process, which are especially luminous regions, and for this reason designated them as luminous cells. It is interesting to note the mention by Okada and Baba of cells with a yellowish tinge when fresh, since yellow cells in the ostracod crustacean, Cypridina, are believed to contain luciferin. The luciferin-luciferase reaction in *Plocamopherus* has not yet been tested but should be investigated at the first opportunity.

Physiology. Little is known of the reflex mechanisms involved in light production by this animal. From the description of successive flashes from slight stimuli we may presume that nerves, whose im-

pulses are controlled by central processes, go to the luminous cells. Hasama (1943) has studied the "electroluminogram" of *Plocamophorus*, the potential change accompanying the light emission. A string galvanometer was used as recording instrument, connected to non-polarizable electrodes, one placed on a dorso-lateral process, the other on the dorsal mid-line, a non-luminous region. Hasama reported a monophasic electrical variation on mechanical stimulation of the animal, although the monophasic aspect of the one record in his paper is not too convincing. The potential lasts about six seconds and very definitely shows signs of diphasic character, falling below the resting potential line before a second stimulus was given. Muscle potentials, described as diphasic, are also found and can be recorded when the animal is not luminescing. Here again the diphasic character is not too apparent. Electrical phenomena may be expected to accompany luminescence when secretion is involved, as in other secretory processes, but the muscle bands, described by Okada and Baba, whose function is to squeeze out the secretion, could be the source of the potential changes in *Plocamophorus*.

Kaloplocamus. The luminous organs of *Kaloplocamus ramosum* from the east coast of Japan have recently been described by Kato (1949). The luminous regions are restricted to the dendrite processes of the head and trunk from which intermittently bluish white flashes of light are emitted. No secretion into the sea water can be detected. The epithelium of the dendrite processes is made up of flat epidermal cells and three kinds of gland cells, mucous, albuminous, and photogenic. The mucous cells, relatively few in number, globular or ovoid, with a pore opening to the exterior, are filled with a secretion which stains in Delafield's hematoxylin and mucicarmine. The albuminous cells are vase-like, also opening by a pore, and filled with a colloidal secretion, staining faintly in eosin. The photogenic cells are more or less elliptical, situated below the epithelium, have no pores and contain very large ($10\ \mu$ diameter) granules, staining black in Heidenhain's hematoxylin and deep red with eosin. The fully developed photogenic cells are large, but can hardly be said to form a light organ. They are innervated by peripheral nerve branches, some of which go to the albuminous cells. Kato has particularly emphasized the fact that the light cells of this species differ greatly from those of *Phyllirhoë bucephala* and *Plocamophorus tilesii*.

Phyllirhoë. The remarkable transparent pelagic nudibranch, *Phyllirhoë bucephala*, has been known since 1807, when it was named by Peron. Since then many naturalists have studied its anatomy and histology, but apparently no one noticed the luminescence until the

work of Panceri in 1872. The family contains two other genera, *Ctilopsis* and *Cephalopyge*, but it is not known if they are luminous.

Phyllirhoë is found in all seas, a small organism $\frac{1}{2}$ to 1 in. long. Panceri's (1873) description of the luminescence is as follows: "if the water in which they are found be agitated, or if they are touched, flashes of light will be seen to come from their body; and if, for the



FIG. 73. *Phyllirhoë bucephala* as it appears by day (left) and at night (right). After Panceri.

purpose of provoking the complete illumination of the phosphorescent elements of the *Phyllirhoë*, one stimulates it with a drop of ammonia, the surface of the body and of the gigantic tentacles shines immediately with a brilliant azure light. The upper and lower edge of the body are the parts where the light is most bright and abundant, so much so that the outline of the animal can be perfectly seen. The light is not communicated to liquids or solids put in contact with it, as happens in many other luminous animals." With a microscope Panceri noted

that "the light escapes from myriads of shining points, which are more or less large and brilliant and more abundant at the upper and lower edges of the animal" [as shown in Fig. 73].

Panceri excluded the chromatophores, containing a "golden yellow" substance, as the source of the light and believed that it came both from rather large spherical cells, called Müller cells by Panceri after their discoverer, each with a nucleus and a yellow refringent spherical body, and also from nerve ganglion cells full of fine granulations and about the same size as the Müller cells. The ganglion cells alone are present in the brightly luminous tentacles while the Müller cells are abundant on the body. Panceri regarded the Müller cells as a special type of nerve cell and claimed to have seen light coming from the esophageal and the tentacular ganglia but this view has not been upheld by later work.

Histology. Vessichelli (1906), Trojan (1910), and Born (1911) have studied the histology with more modern methods. Vessichelli made no special mention of luminescence except to say the Müller cells were the phosphorescent cells, and Born did not study living specimens. Trojan's work is based on observations of living specimens as well as fixed material. He placed the source of the light in mucous cells scattered over the epithelium, not in the Müller or ganglion cells as Panceri had supposed.

In addition to the mucous cells of the epithelium, Trojan (1910) has also described mucous glands, albuminous gland cells, chromatophores that function through contraction of muscle fibers as in cephalopods, and sensory cells. The albuminous and mucous cells are reproduced in Fig. 74.

Careful observation of *Phyllirhoë* reveals two types of luminescence, a weak diffuse light over the whole body, including the tentacles, and a bright luminescence, restricted to points, generally not in the tentacles or tail region and more abundant in the posterior third than the anterior or middle third of the body. The diffuse light, according to Trojan, comes from mucous cells and the points of light from the secretion of mucous glands, since he classed the luminescence as extracellular and extraglandular. Nerve fibers were found to end on the mucous cells.

On the other hand, Born (1911) doubted that the mucous cells are the photogenic ones, since they are abundant in many non luminous nudibranchs, and their role is probably the secretion of a mucus whose function is protective in the absence of a shell. He also considered it unlikely that the many celled glands with their acidophilous contents could have developed from single mucous gland cells, but was unable to

add any definite evidence to clear up this point or to prove with certainty which cells are photogenic. Dahlgren (1916) has suggested that the mucous or photogenic cells of Trojan, which stain in iron hemotoxylin, secrete luciferin and the eosinophil albuminous cells secrete luciferase, while Hoffmann (1939) has taken the position that the Müller cells are undoubtedly the photocytes, but that they are not nerve cells as Panceri had supposed. It is obvious that further investigation is necessary.

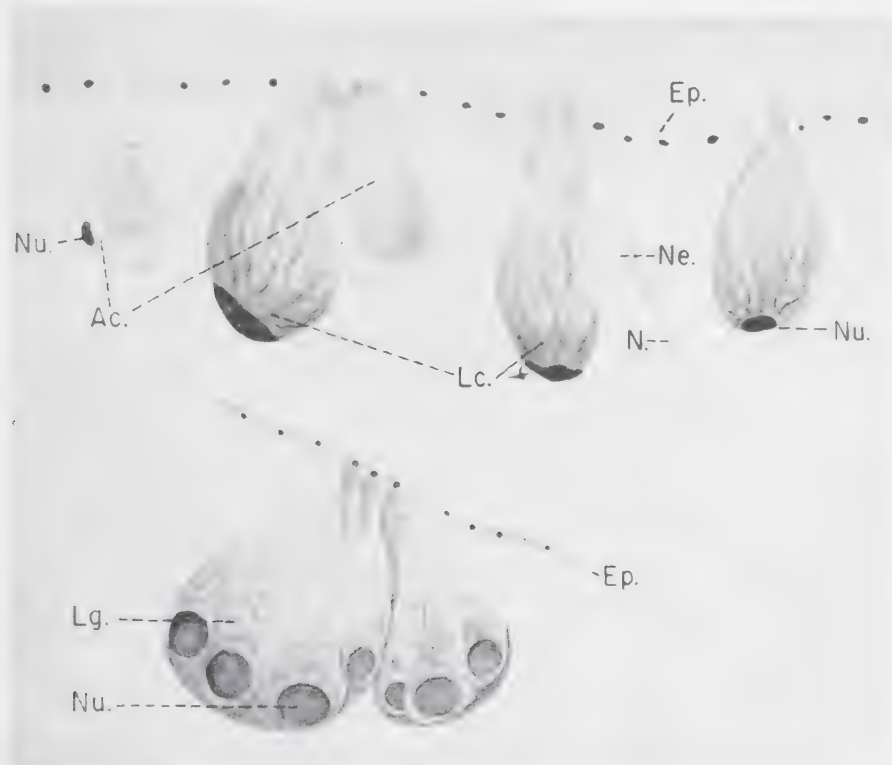


FIG. 74. Luminous cells (Lc) from the body epithelium (Ep) of *Phyllirrhoe bucephala*. Ac, albumen cells; Lg, light gland; N nerve with ending, Ne; Nu, nuclei. After Trojan and Dahlgren.

Physiology. Panceri observed chemical stimulation, especially with ammonia or in distilled water, but was unable to stimulate *Phyllirrhoe* electrically. However, Trojan found that when electrodes were applied and the current made, the whole animal luminesced rather slowly; when broken, the light diminished slowly. The light also disappeared after some three minutes, even if the current was passing, but after a rest, luminescence again resulted from further stimulation. The light was not steady but quivering and came from points irregularly scattered over the body except on the tentacles. Trojan also remarked

on the ready stimulation of luminescence by ammonia pointing out that the luminescence of *Phyllirhoë* could not be due to luminous bacteria since their light is quenched by ammonia. Distilled water also extinguishes most bacterial light immediately.

Panceri found that animals in sea water which is slowly heated become spontaneously luminous at 44°C. and the light persisted, although dim, to 61°C. Sunlight had no effect on the ability to luminesce.

Biochemistry. No modern studies of the chemistry of light production in *Phyllirhoë* have been made. It is not known if the luciferin-luciferase reaction can be demonstrated or whether the luciferin of *Pholas* will react with the luciferase of *Phyllirrhoë* or vice versa. Panceri attempted making extracts as follows:

"With the intention of trying whether the luminous matter could be extracted from the animal I pressed some individuals in a cloth and there escaped from them, as they were shining, a few drops of a bright liquid, which became dark immediately, but fresh water soon made it shine afresh.

"With the object of observing whether light could be obtained even after the death of the animal, I let a *Phyllirhoë* dry quite naturally in a glass. The following day the animal was already dry and fragile and I thought of wetting it again with fresh water. By rubbing it with the finger, I saw that the water that dropped from it began to shine with a uniform dim light. As the animal absorbed the water, it became detached from the glass, and then, in pressing and rubbing it between my fingers, dipped into fresh water, a luminous matter began to spread, very like a pale cloud, which, as it dilated, soon illuminated the whole vessel."

Panceri's observation that a non-luminous extract of the animal will again emit light when fresh water is added recalls the behavior of extracts of medusae, pennatulids, and ctenophores previously described. Tests for *Phyllirhoë* luciferin and luciferase, as well as the action of adenosine triphosphate are urgently needed in order more properly to characterize the chemical nature of the luminescence in this form.

Use of Light. *Phyllirhoë* is a typical example of many transparent pelagic plankton animals, some of which are luminous and some are not. Why *Phyllirhoë* should emit light presents the same problem as the luminescence of transparent *Tomopteris* or jelly-fish or ctenophores. The answer is uncertain. Trojan has suggested that *Phyllirhoë* may be poisonous and that the light is a warning to scare away predacious forms, similar to the defense coloration of many pigmented animals. No evidence for this view has been obtained and the whole problem remains unsettled.

GASTROPODA PROSOBRANCHIATA

Doliacea

There is one reference in the literature to a luminous marine gastropod. R. D. Turner, who recently (1948) published a paper on "The family Tonnidae in the Western Atlantic," has informed me by letter that the following paragraph⁵ was inadvertently omitted from the publication:

"It appears that *Tonna galea* Linné is luminescent. Mr. Harry Hurst of Puerto Plata, Hispaniola, has written that this species can be seen at night at some distance. When the animal is moving about with the foot well extended, it gives off a greenish-white light which appears strongest at a distance but is barely visible when one is standing directly over the specimen."

It would be most interesting to pursue this matter further, as the Tonnidae or Doliidae have not been previously reported to contain luminous species. They belong to the tribe Doliacea, related to the Heteropoda, also reported to be luminous. A brief classification by J. Thiele of this enormous group is as follows:

Order 1 Archeogastropoda (5 tribes and 18 families)

Order 2 Mesogastropoda (15 tribes and 60 families)

Tribe 13 Heteropoda (3 families)

Atlantidae (3 genera)

Carinariidae (3 genera)

Pterotracheidae (*Pterotrachea*, *Firoloida*)

Tribe 14 Strombacea (4 families, 8 genera)

Tribe 15 Doliacea (5 families, 16 genera)

Oocorytidae (Oocorys)

Cassididae (4 genera)

Cymatiidae (7 genera)

Bursidae (Bursa = *Ranella*, *Bufo*)

Doliidae or *Tonnidae* (*Dolium* = *Tonna*, *Pirula*)

Order 3 Stenoglossa (4 tribes and 16 families)

Heteropoda

Concerning luminous heteropods, the information is meager. Keferstein made the following statement in the first edition of Bronn's *Klassen und Ordnung des Thierreichs*.⁶ "Heteropoda contribute to the phosphorescence of the sea and among *Pterotracheates* I have myself admired the beautiful bluish light emitted at the slightest stimulation, especially from *Nucleus*." This record of *Nucleus* luminescence has

⁵ The paragraph will appear at the end of Vol. 2 of *Johnsonia*.

⁶ Vol. 3, 2 abt., p. 839, 1862-66.

been mentioned by Simroth in a later treatment of prosobranchiate gastropods in Bronn's *Thierreichs*⁷ and by Steuer⁸ and Mangold (1910). In the list of luminous mulluscs, compiled by Grimpe and Hoffmann (1930) for *Tabulae Biologicae*, the heteropod, *Pterotrachea* sp. is included, but no reference is given. Thiele's (1931) *Handbuch der Systematische Weichtierkunde* contains no *Nucleus* in the index, but in a figure of *Pterotrachea*, the "Nucleus" is shown as a structure composed of liver and gonad. Although *Nucleus* is listed in Neave's *Nomenclator Zoologicus* for 1940, it is possible that Keferstein indicated *Nucleus* as the luminous region and that *Pterotrachea* is the genus. There appear to be no recent records of luminescence in *Pterotrachea*.

GASTROPODA PULMONATA

From time to time accounts of luminous snails (*Helix*) have appeared in the literature. In one of these cases, observed by Newall (1879) and reported in *Nature*, the snail, 1½ in. in length, had swallowed a glow-worm, and Newall could see the light shining inside. The observation was doubted by McLachlan (1879), who pointed out that glow-worms usually eat snails, but Newall in a reply said there was no doubt of the fact.

True self-luminescence is hardly to be expected among the terrestrial gastropods, but a most interesting case has recently been reported by Haneda (1946) in *Dyakia striata* of the Zonitidae⁹ from the Malay Peninsula. Still more unusual, since it represents a striking exception to the rule that only salt water animals are luminous, is the fresh water snail or limpet, *Latia neritoides* of the family Ancyliidae,⁹ from New Zealand, first described as luminous by Suter in 1890 and figured in his *Manual of the New Zealand Mollusca* in 1913. The position of these families in a classification⁹ of the pulmonates by J. Thiele is as follows:

Pulmonata

Basommatophora (Mostly fresh water, a few land or marine) 4 tribes and 11 families

Ellobiidae (4 subfamilies, 12 genera)

Otinidae (Otina)

Amphibolidae (Amphibola, Ampularina)

Gadiniidae (Gadinia)

Siphonariidae (Siphonaria)

Chiliniidae (Chilina)

⁷ Vol. 3, 3 abt., p. 983, 1896-1907.

⁸ Steuer, A. *Leitfaden der Planktonkunde*, Leipzig, 1911, p. 165.

⁹ In Thiele's *Handbuch der systematische Weichtierkunde*, *Dyakia* is placed in the Ariophantidae and *Latia* in the Latiidae.

*Latiidae (Latia)*¹⁰

Physidae (Aplexa, Physa)

Lymnaeidae (Lymnaea, Amphipeplea)

Planorbidae (9 genera)

Anchylidae (9 genera, including Gundlachia)

Stylommatophora (Land snails) 14 tribes and 50 families including

Zonitidae (Striatura, Mesomphix, Gastrodonta, Lyrodiscus, Aegopina, Zonites,

Zonitoides, Vitrea, Oxychilus = Hyalinia) and

Ariophantidae (128 genera, including *Dyakia*)

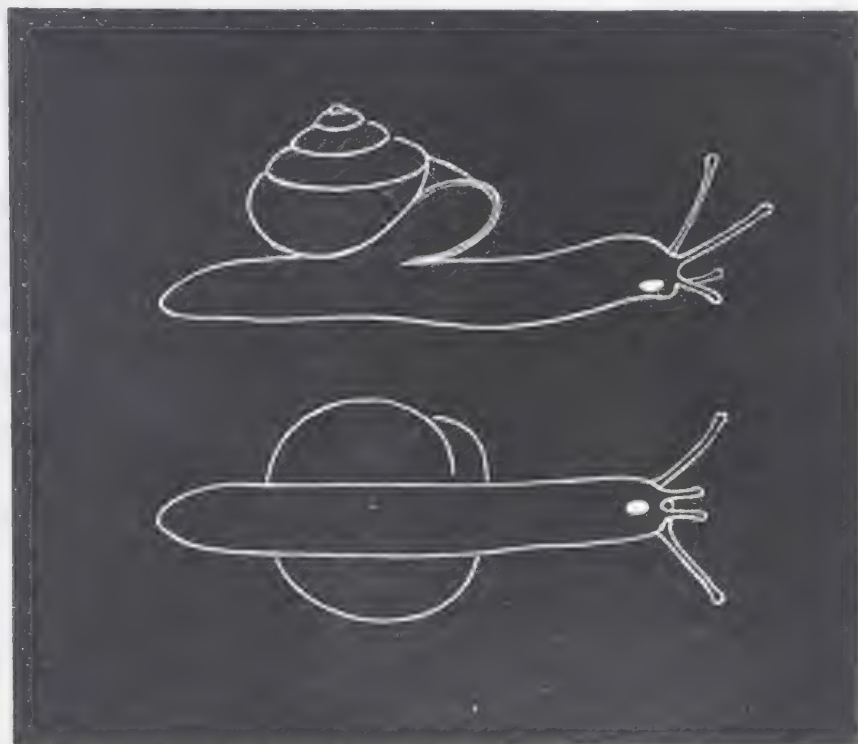


FIG. 75. Diagram of the snail, *Dyakia striata*, to show luminous organ (white dot near tentacles). After Haneda.

Dyakia striata

This snail, about 15 mm diameter, which lives on leaves and grass in the Malay Peninsula, is another example of a luminous animal whose ability to light has escaped most students of bioluminescence. Haneda (1948) has made a detailed study of specimens obtained in Singapore and Kuala Lumpur in 1943. As shown in Fig. 75, the light appears inside the anterior region of the foot and cannot be seen when the animal is stimulated and has withdrawn within its shell. Observed through the head or transparent muscles of the foot, the lumi-

¹⁰ Suter (1913) placed *Latia* in the Anchylidae, together with *Gundlachia*

nescence is bluish white and flickers like a fire fly, but the time relations of the flashing depend on the stage of development of the snail and various conditions. Young snails immediately after hatching emit a weak, apparently continuous, luminescence over the entire foot, but on closer inspection the diffuse glow can be resolved into small flashes scattered over the area. Later in development the light condenses to the oval luminous organ just below the mouth. As the animal grows, the flicker rate diminishes, and some individuals do not luminesce at all. The normal duration of a flash at 25°C is two to three seconds. The animal flashes two, three, or four times and then pauses for thirty to sixty seconds, when another short period of flashing begins. The intensity of each flash is the same and very bright, visible in electric light if shaded by the hand.

The luminescence comes from gland cells and not from luminous bacteria. These large luminous gland cells are below the mucous gland of the foot and surround its opening, but no luminescent material is secreted to the outside, the light being intracellular. When alive the gland cells are pale yellow-green and in sections can be readily distinguished from the other smaller cells of the region.

Latia neritoides

These fresh water limpets, shown in Fig. 76, have rather small oval seed-like shells, with dimensions around 8.5 x 6 x 3 mm high, black in color, and quite inconspicuous as they cling to stones in lakes, rivers, and especially swift-running streams throughout the North Island of New Zealand. Suter (1890) first reported on specimens he had found in Kaiwara Creek near Wellington and was astounded to find: "all the animals highly phosphorescent with a violet light, and this was intensified by a touch with a needle. The secreted mucus was also phosphorescent for some time." He said he knew of no other luminous fresh water mollusc and hoped to examine them more fully later, but apparently never did so.

Suter originally referred to the limpet as *Latia lateralis* and remarked that it would be interesting to see if *L. fluviatilis* was luminous. At present only one species is recognized, *L. neritoides*, a name given by Gray in 1850. Although the luminescence of *Latia* is mentioned by Simroth and Hoffmann in Bronn's "Thierreichs," all the authors of general works on bioluminescence have overlooked it, a rather surprising omission in view of the fact that *Latia* is fresh water luminous animal. The family Ancyliidae contains another genus, *Gundlachia*, of wider distribution than *Latia*, which is confined to New Zealand, but no reports of a luminous *Gundlachia* have appeared.

The author has received specimens dried over CaCl_2 from Mr. B. J. Bowen of Auckland, New Zealand. When these animals were ground in a mortar and moistened with water, the luminescence appeared. Bowen has written in a letter as follows: "The luminescence [in life] is very vivid. If a few specimens are placed in a bottle they can readily be seen in the dark as green ovals (i.e., the light comes mainly from the cavity surrounding the foot). If the bottle is shaken they become brilliantly luminescent and exude trails of mucus which emits a strong greenish light. Half a dozen will provide plenty of light to read newsprint by. The light from the mucus rapidly fades. . . ."

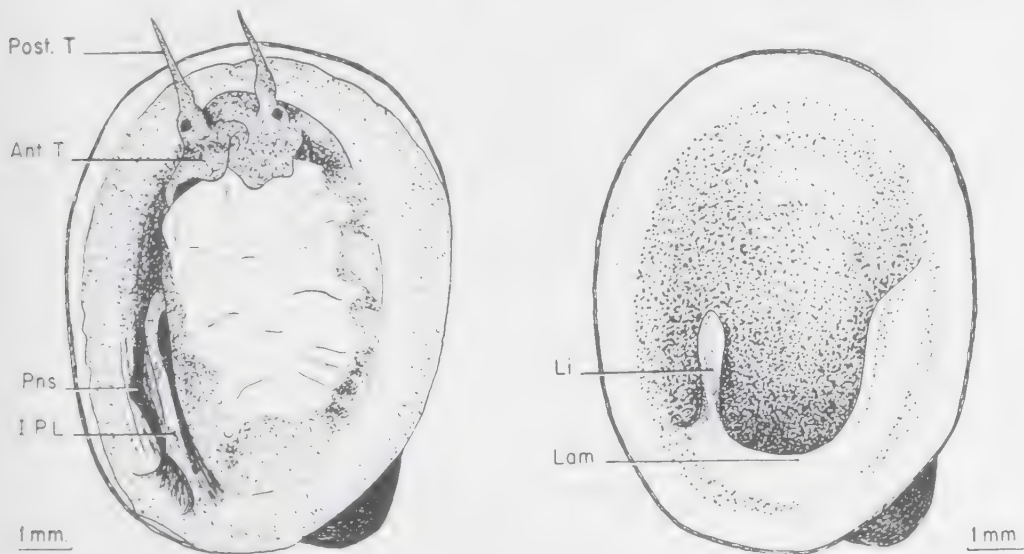


FIG. 76. Left. *Latia neritoides* under surface, showing posterior and anterior tentacles (Post. T. and Ant. T.), pneumostome (Pns), inferior pallial lobe (I.P.L.), lingula (Li), and lamella (Lam). On right the animal has been removed and the shell interior exposed. After Bowen.

Bowen (1950) obtained the luciferin-luciferase reaction on mixing a hot water (70°C) extract of crushed *Latia* (after cooling) with cold water containing mucus whose luminescence had disappeared. The possibility that cytolysis or granulolysis of mucous material was responsible for the luminescence on mixing was tested by adding ether to the cold mucous solution, but no luminescence resulted as in the case with ctenophore extracts in which granulolysis plays an important part in light production.

In a careful histological study Bowen (1950) has mapped the luminous regions and described the photogenic cells of *Latia*. The luminescent trails of mucus appear in the groove between the foot and the mantle, coming from regions characterized by many large mucous ($200 \times 40 \mu$) and granular ($150 \times 30 \mu$) cells lying below the cuboidal

epithelium in a loose connective tissue containing branching melanophores and muscle fibers. These regions are the surface of the head, the anterior tentacles, the lateral surfaces of the foot, the inferior pallial lobe, and the free surface of the mantle, but not of the pulmonary cavity. That the mucous and granular cells are involved in light production is indicated by the fact that they are absent from related

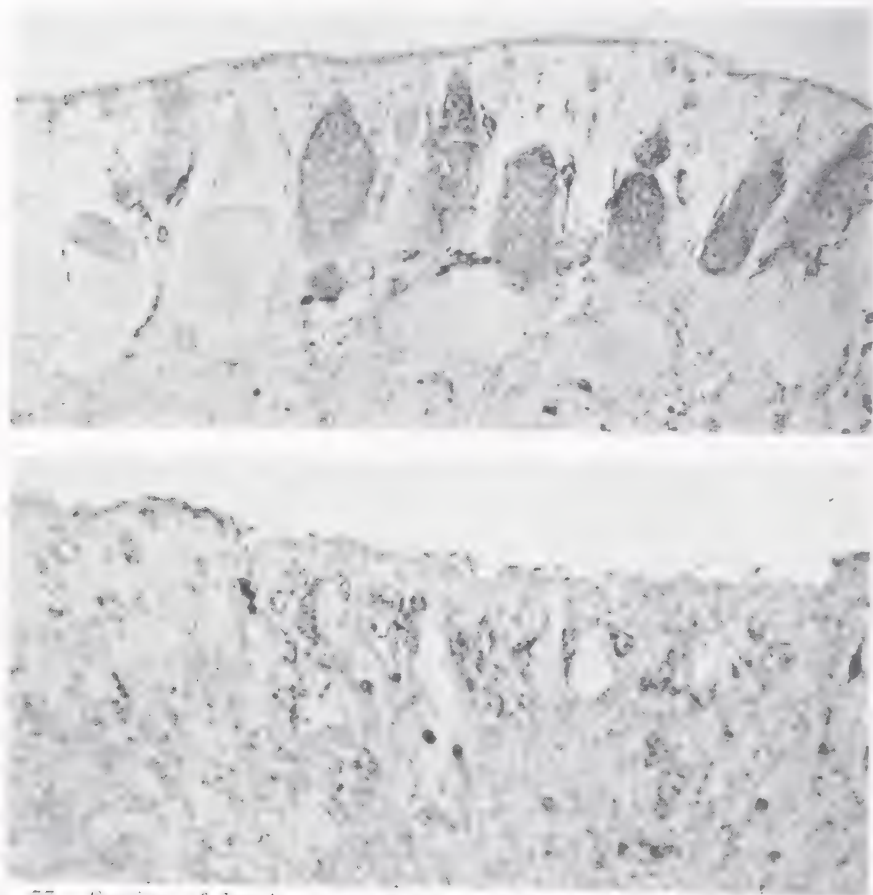


FIG. 77. Section of luminous epithelium of *Latia*, showing large light mucous and smaller dark granular cells, before (above) and after (below) the animal has been stimulated to exhaustion. After Bowen.

non luminous molluscs, like *Gundlachia lucasi*. Similar mucous and granular cells are characteristic of other luminous molluscs.

After stimulation, these types of cells lose their contents and practically disappear, most of the cells going to form the luminous secretion. The appearance of the mucous and granular cells is shown in Fig. 77, before and after prolonged stimulation. It would be satisfying to be able to state that one type of cells contains luciferin and the other luciferase, but the evidence is at present lacking.

Regarding the use of the light, little can be said. Bowen has discussed this question and has come to the conclusion that it is not a lure for food as the snail is herbivorous; not a sex lure as the snails are very numerous and hermaphrodite, and the light can be emitted the year round; possibly a warning signal, although *Latia* is eaten by both trout and eels and appears to be quite palatable. The function is still unknown.

PELECYPODA OR BIVALVIA

Despite the large number of species (some 11,000) in this group of molluscs with hinged shells, only the Pholadidae and Gastrochaenidae, containing *Pholas dactylus* and *Rocellaria* (*Gastrochaena*) *grandis*, are markedly luminous. The allied species, *Pholas* (*Barnea*) *Candida*, according to Okada (1927), produces a weak light while *Pholas costata* and many others are not luminous. *Pholas dactylus* has been a classic animal for the study of bioluminescence and a model for many of the experiments on the chemistry of light production.

The position of the Pholadidae in a classification of the bivalves is indicated in the following classification of J. Thiele. Luminous genera are in italics.

Bivalvia

Anisomyaria (11 families)

Taxodonta (6 families)

Eulamellibranchiata

Schizodonta (8 families)

Heterodonta (24 families)

Hemidapedonta (5 families)

Desmodonta (9 families)

Mesodesmatidae (*Mesodesma*, *Davila*, *Ervilia*)

Mactridae (6 genera)

Cardiliidae (*Cardilia*)

Saxicavidae (*Panomya*, *Panopea*, *Cyrtodaria*, *Saxicava*)

Myidae (*Mya*, *Tugonia*, *Sphenia*)

Corbulidae (*Corbula*)

Gastrochaenidae (*Gastrochaena* = *Rocellaria*, *Fistulana*)

Pholadidae (*Pholas*, *Zirphaea*, *Barnea*, *Pholadidea*, *Talone*, *Martesia*,

Paraphols, *Jouannetia*, *Xylophaga*)

Teredinidae (*Teredo*, *Bactronophorus*, *Tachsia*, *Bankia*)

Anomalodesmata (12 families)

Pholas dactylus

The boring mollusc, *Pholas dactylus*, shown in Fig. 78, has been known from antiquity. Aristotle did not mention the luminescence, but Pliny devoted some space to it. The generic name, *Pholas*, comes

from the Greek, *pholas*, meaning hidden or lurking in a hole, because of the habit of the mollusc of boring into soft rock and hiding there, with only the siphon extruded. The specific name, *dactylus*, is also from the Greek *daktylos*, the forefinger, since the shape of the animal somewhat resembles a finger. The French call them dails or pholads, the English, piddocks, and the Germans, Bohrmuschels or boring mussels. They have been a favorite article of food since classic times.

Although luminescence of *Pholas* was also known to the various compilers of the sixteenth and seventeenth centuries¹¹—Rondelet, Boussuet, Aldrovandi, Gesner, Kircher, Olaus Magnus, etc.—the basic experimental study was undertaken in both France and Italy, early



FIG. 78. The boring mollusc, *Pholas dactylus*, opened to show the luminous regions, a, b, and c. After Panceri.

in the eighteenth century. Réaumur, the great French physicist and naturalist, contributed a memoir to the Royal Academy of Sciences in Paris in 1723, entitled, *Des merveilles des dails ou de la lumière qu'ils répandent*. Réaumur mentioned Pliny's statements and confirmed many of them—the fact that the luminous humors illuminated the mouth of those who ate and the hands of those who touched the dails and that luminous drops came from them. He also noted that only the animal, not the shell, was luminous, but erred in stating that all parts emitted light, even the flesh inside, when cut. He was the first to show that dried Pholads will luminesce again when moistened and studied the effect of other liquids, fresh and salt water. He also tested "eau de vie," which put out the light. Réaumur remarked that, contrary to the case of luminous fish, which develop luminescence only when they decay (due to luminous bacteria), the dail luminesced

It is interesting to note that Bartolin in 1643 in "*De Luce Animalium*" never saw the light of *Pholas*, thinking it an invention of Pliny.

more brightly the fresher it was; he compared the dail to the glow-worm and luminous centipedes.

In the next year, 1724, several communications by Beccari, Monti, and Galeati to the Bologna Academy of Science dealt with pholads. These men experimented with the animals themselves and with water rendered luminous by *Pholas*, testing the effect of temperature and the addition of various chemicals. Priestley, in his book on the History of Vision, Light and Colours, etc., published in 1772, has given an excellent account. "Of all the liquors to which he [Beccarius] put the pholades, milk was rendered the most luminous. A single pholas made seven ounces of milk so luminous that the faces of persons might be distinguished by it and it looked as if it were transparent.

"Air appeared to be necessary to this light; for when Beccarius put the luminous milk into glass tubes, no agitation would make it shine, unless bubbles of air were mixed with it. Also Montius and Galeatius found that, in an exhausted receiver, the pholas lost its light, but the water was sometimes made more luminous, which they ascribed to the rising of bubbles of air through it.

"Beccarius, as well as Réaumur, had many schemes to render the light of these pholades permanent. For this purpose he kneaded the juice into a kind of paste, with flour, and found that it would give light when it was immersed in warm water; but it answered best to preserve the fish in honey. In any other method of preservation the property of becoming luminous would not continue longer than six months, but in honey it had lasted above a year; and then it would, when plunged in warm water, give as much light as ever it had done."

It will thus be seen that these men laid the foundation for much of the later chemical work. They established the important necessity of oxygen (air) for luminescence and showed how material could be preserved, by drying and other means, a most valuable contribution for chemical study.

In 1791 Poli published a beautiful figure of *Pholas dactylus*, showing all five light organs, but he did not appear to have realized their luminous character. The modern study of the luminescence begins with another Italian, Panceri (1872, 73) whose classic diagram (see Fig. 80) of the animal indicates the five luminous glandular regions, a line on the superior edge of the mantle, the paired cords on the siphon and the paired triangles, now frequently referred to as the organs of Poli. Panceri studied *Pholas* with his usual care and carried out biochemical and physical experiments on the luminescent process. He found no heat whatever to accompany the luminescence, at least none that could be detected with the same sensitive thermopiles and galva-

nometer that had been used by Melloni in detecting the heat of lunar rays. Spectroscopically the light was a narrow continuous band in the blue region, like that of other luminous animals he had examined.

It was Raphael Dubois who made the most extensive study of the animal. Over a period of some forty years Dubois has published a series of papers dealing largely with the biochemical aspects of light production, summed up in his article (1928) on "Lumière" in Richer's *Dictionnaire de Physiologie*. Dubois' principal work on *Pholas* (1892) is a monograph of 167 pages and 15 plates, *Anatomie et Physiologie de la Pholade Dactyle*, divided into four sections: I. Morphologie, II. Physiologie zoologique, III. Physiologie comparée, and IV. Photogénie. Dubois had previously (1887) demonstrated the existence of luciferin and luciferase in *Pholas* and had also been able to isolate luminous bacteria, called *Bacillus Pholas* (1888) or *Bacterium Pholas* (1889) which he at first thought were the source of the light but later (1890) retracted the suggestion and emphasized the fact that in *Pholas*, photogenic function was independent of cellular activity, although the photogenic substance behaved like living protoplasm. Luciferase was called a zymase or a "ferment figuré" and was identified with small organized bodies of "granulides" which are conspicuous in most luminous animals. The work of other investigators has been chiefly on the histology of the luminous regions.

Histology. The minute structure of the luminous regions of *Pholas* has been studied with considerable care. Six investigators, Panceri (1872), Rawitz (1892), Dubois (1892, 1916), Förster (1914), Dahlgren (1916), and Okada (1927), have published on the subject. Panceri (1872) appears to have been the first to observe the secretion of *Pholas* microscopically, describing the furrowed mantle as covered with a ciliated epithelium of special structure and the light organs as containing groups of fragile cells which easily lose their contents "of very fine granulations, of small greasy drops, and even of granular masses" which mix with the mucus and render the sea water luminous. He figured these granules and also sections of both triangles and cords and noted that they received a nerve supply.

Rawitz' (1892) paper is mostly confirmatory, and the monograph of Dubois contains drawings of individual gland cells of the luminous epithelium, of whitish appearance in the fresh state, whose contents, the "granulides," are extruded on nerve stimulation. Dubois also described other granular cells of pear and bottle shape, deeper in the epithelium, which he at first considered to be mucous cells, but later believed to be "éléments migrants," corresponding to the *clasmotocytes* of Ranvier.

Förster (1914) has given by far the best account of the histology. The structure of all five luminous regions is practically the same. Under the thin columnar ciliated epithelium, there are two kinds of gland cells, a layer of mucous cells, staining in thionin and mucicarmine near the epithelium, and below this a thicker layer of "luminous" cells with large granules, staining in iron hematoxylin. These cells are $40\ \mu$ long and $30\ \mu$ wide, while their long narrow ducts may be 50 to $450\ \mu$ long in order to reach the surface of the epithelium between the layer of mucous cells, which also have short but definite ducts. Förster found nerves branching to fine twigs and entering the luminous organ, but was unable to locate the exact position of the endings. He did not mention luciferin or luciferase, but regards the deep layer of large cells filled with granules and with long ducts as the photogenic cells, since they are absent in non-luminous parts of *Pholas*, while the mucous cells are scattered over the entire mantle and outer surface of the siphon.

Dahlgren (1916) has presented the same picture of the histology of the light organ of *Pholas* but raised the question as to the function of Förster's mucous cells, whether they secrete only mucous or whether they may not also secrete luciferase, while the deep layer of luminous cells with the large prominent granules may secrete luciferin. Dahlgren found true mucous cells in other non-luminous parts of the epithelium, but they did not stain quite like Förster's mucous cells of the luminous organ. His drawing is reproduced as Fig. 79.

Finally, Okada (1927) has studied *Pholas dactylus*, as well as two related species, *Pholas (Barnea) candida* and *Pholas (Zirphaea) cristata*, but has published no figures of sections. The two last species also contain cells similar to the "mucous" and "luminous" cells of Förster, although their distribution and state of development are different. Although *Pholas (Barnea) candida* is usually considered non-luminous, Okada noted a weak momentary luminescence of this form, at the time the mantle was opened. The light arose from the region of the triangular organs which contain "luminous" cells of smaller size than those of *P. dactylus*. Granular cells, almost like the photogenic cells of *P. dactylus* but staining less well in hematoxylin are also present around the posterior opening of the mantle, but no luminescence appears there.

In *Pholas (Zirphaea) cristata*, the surface of the mantle is covered with eosinophil cells, and there is some development of granular cells, but no sign of the five luminous regions of *P. dactylus* and no luminosity can be evoked from this form.

The similarity in histological structure of cells in luminous and non-

luminous pholads and in luminous and non-luminous regions of *P. dactylus* led Okada to believe that a very small change in the constitution of the granules results in the ability to emit light. He suggested that luminous species probably arise by a process of mutation, with a more or less definite series in the degree of formation of luminous organs, exemplified by *Pholas cristata*, *P. candida* and *P. dactylus*.

Biochemistry. The successful attempts of early workers to retain the light of *Pholas* for future display by drying and by chemical meth-

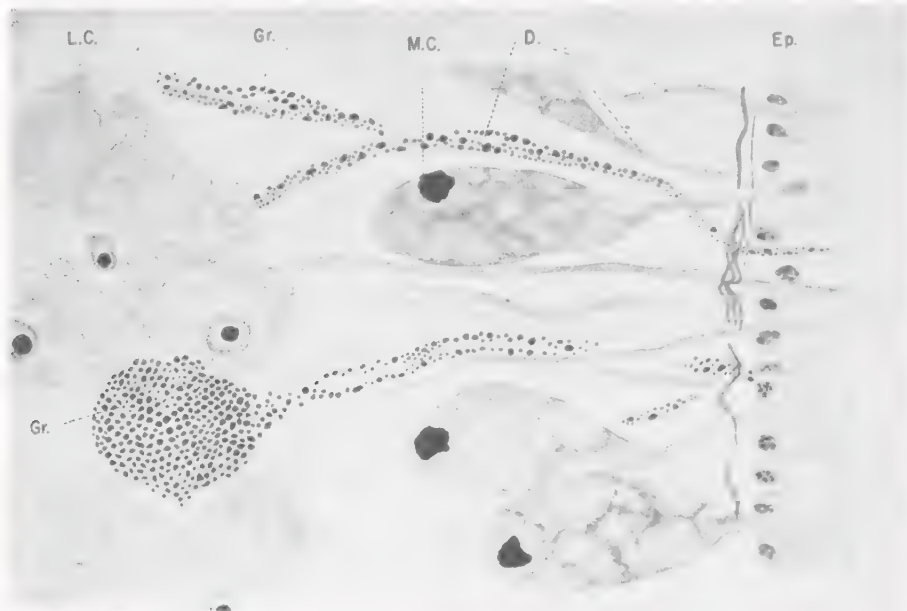


FIG. 79. Section of the luminous tissue of *Pholas*, showing epithelium (Ep); M.C., mucous cells; L.C., luminous cells, with granules (Gr) in cells and ducts. D. After Dahlgren.

ods has already been mentioned. Panceri's (1872, 73) work was largely with fresh material, the luminous matter that could be spread on a sheet of paper. He found that this material became no brighter in pure oxygen, but did gradually lose its light in CO_2 , returning when air was again admitted. Sunlight had no effect on the intensity but distilled water, alcohol, and ether momentarily excited its luminescence. Panceri wrote, "The luminous matter contained in the cells of the luminous epithelium is soluble in alcohol and ether, and it would be very important for chemists to study it closely, so much the more so as it is easy to procure where *Pholades* are abundant."

Whether Dubois was influenced by this statement is unknown, but he did devote a large part of his study of *Pholas* to biochemical experiments. Like Beccari, Dubois recommended honey or sugar as the most

successful means of keeping luminous material and years ago the author was able to repeat many of Dubois' experiments, using sugar preserved siphons sent by him. *Pholas* is most famous because of its association with the names, luciférine and luciférase.¹² The idea of an oxidizable compound, luciferin, and an oxidizing enzyme, luciferase, occurred to Dubois (1885) from experiments on the elaterid beetle, *Pyrophorus*, but *Pholas* supplied the material for detailed study of their chemical properties over a period of many years. Without going into the history of all the findings which have been summarized from time to time, (Dubois, 1913, 14, 17, 28) it is sufficient to indicate their mode of preparation and properties.

In the luminous epithelium or luminous extracts of *Pholas*, both luciferin and luciferase are present. If this extract is allowed to stand until the light disappears, all the luciferin will be oxidized and the luciferase will remain, giving a luciferase solution. Like all enzymes luciferase is destroyed on heating¹³ so that if the luminous extract is heated, the luciferase is denatured and a luciferin solution remains. When cool, this luciferin solution will again luminesce, if some luciferase is added to it.

Dubois (1914) has listed the properties¹⁴ of these substances as follows: *Pholas* luciferin is destroyed above 70°C; dialyzes slowly, oxidizes with light production in the presence of *Pholas* luciferase, potassium permanganate, hydrogen peroxide, haematine and hydrogen peroxide, barium or lead peroxide, hypochlorites and the blood of various marine molluscs and crustacea. It is insoluble in fat solvents, but forms a colloidal solution in water from which it is precipitated unchanged by picric acid, alcohol, and ammonium sulphate. It is not precipitated by sodium chloride or acetic or carbonic acids, except in presence of neutral salts. It forms an insoluble "alkali albumin" with ammonium hydroxide, which increases the luminescence. At various times Dubois has regarded luciferin as a proteose, as a nucleoprotein, and as a natural albumin having acid properties. It occurs only in the luminous parts of *Pholas*, not in non-luminous animals.

Dubois found that *Pholas* luciferase has all the properties of an enzyme, is destroyed at 60°C, is non-dialyzable and insoluble in fat solvents, but forms a colloidal solution in water. It is not affected by

¹²The English equivalent, luciferin and luciferase, will be used throughout this book.

¹³In early papers Dubois spoke of boiling the extract, but in later ones of heating to 70°C.

¹⁴The author is listing the properties as given by Dubois. It is very probable that work with purified material may greatly change the statements expressed here.

1% sodium peroxide but its activity is suspended in saturated salt solutions, sugar, or glycerine, and returns on dilution. It is digested by trypsin and is slowly destroyed by the fat solvent anesthetics, such as chloroform. Because he found iron in an extract of *Pholas*, dialyzed for a long time against running water, Dubois considered that luciferase is associated with iron and reported that it will oxidize the ordinary oxidase reagents, such as pyrogallol, gum guaiac (feebly), α -naphthol, and paraphenylenediamine. It remains to be proved, however, that luciferase, and not the oxidizing systems such as occur in all cells, is responsible for the coloration of these reagents. Dubois regarded the enzyme as a special kind of oxidase, comparable to the oxydons of Batelli and Stern and called it a carrier (*emprunteur*) of oxygen in his latest (1928) paper. He found luciferase or substances capable of giving light with *Pholas* luciferin in the blood of many non-luminous crustacea and molluscs.

In addition to luciferin and luciferase, Dubois has described a number of other accessory luminous substances. It is obvious that luciferin must be formed from some precursor in the cell, and following the usual biochemical terminology, Dubois called it proluciferine or preluciferine, and believed that it is converted into luciferin by another enzyme, coluciferase.¹⁵

The reader must consult Dubois's papers (1907, 17, 18) for details of properties and preparation of these substances. It is sufficient to say that the mode of preparation both of *Pholas* proluciferin and *Pholas* coluciferase was such as might be used in the preparation of an oxidized luciferin, and in later papers Dubois (1919, 20) took the view that his coluciferase was a reducing enzyme or a hydrogenase which formed luciferin by reduction from an oxidized form. He stated that a *Pholas* luciferase solution heated to 65°C had the ability to reduce saccharose with formation of bubbles of hydrogen.

In his most recent work, Dubois (1928) has summed up his ideas of the biochemistry of *Pholas* luminescence, retaining the terms preluciferin, luciferin, luciferase, and coluciferase, and introducing also the terms oxyluciferine and peroxy-luciferine. Peroxy-luciferin is the complete oxidation product of luciferin, what the author (1918) has referred to as oxyluciferin in the luminescence of the ostracod crustacean, *Cypridina*.

It is probable that the luminescent reaction in *Pholas* is complicated by the stability of the photogenic granules (*vacuolides* of Dubois

¹⁵ Dubois's use of the word coluciferase does not conform with modern usage. Gerretsen (1920) has called the enzyme that forms the photogen, photogenase, and believes the photogen is then oxidized by an oxidase.

observed by all workers. Anything which dissolves these granules results in light emission. A sea water extract of the siphons of *Pholas* allowed to stand until the luminescence disappears will again emit light on dilution with distilled water, on slow heating, or addition of ether, chloroform, saponin, or sodium glycocholate. These are all cytolytic effects and the luminescence is due to solution of granules. These granules can be seen to dissolve under the high power of the microscope if the luminous organs are teased on a slide and a little saponin powder is added. The solution of the numerous photogenic granules takes place like a flash, and the resulting solution of the granules still contains luciferase, for it will react with *Pholas* luciferin and emit light—the true luciferin-luciferase reaction. Drying the luminous tissue of *Pholas* will also abolish the luminescence due to granulolysis. All attempts to demonstrate interaction of *Pholas* luciferase with *Cypridina* luciferin and *Cypridina* luciferase with *Pholas* luciferin have been negative.

A most important experiment yet to be carried out is the addition of adenosine triphosphate (ATP) to *Pholas* extracts to determine if the luminescence can be revived by this high-energy phosphate compound, as in the case of the fire-fly. The action of ATP may modify present ideas of the *Pholas* luciferin-luciferase reaction and the role of non-luminous extracts in causing luminescence of *Pholas* luciferase as it has in the fire-fly.

The author observed no particular fluorescence in the luminous regions of *Pholas* in ultraviolet light and has confirmed the necessity of oxygen for luminescence of *Pholas*, as well as the insensitivity of this mollusc to inhibition of luminescence by sunlight, first demonstrated by Panceri.

Use of Light. Little more can be said regarding the purpose of the light of *Pholas* than was said for *Chaetopterus*. Puffs of luminescent slime have been seen to emerge from the siphon when *Pholas* is disturbed and the best guess is that the light masses scare away predacious animals.

Rocellaria grandis

For many years it was thought that *Pholas dactylus* was the only luminous species among the lamellibranchs, but Haneda (1939) has recently described another bivalve, *Rocellaria* (*Gastrochaena*) *grandis* from the reefs of the Palau islands, which also produces a luminous secretion. His figure shows a mollusc like *Pholas* in general appearance, boring in coral rock on the reefs. However, unlike *Pholas*, *Rocellaria* has only a luminous region on the mantle and paired

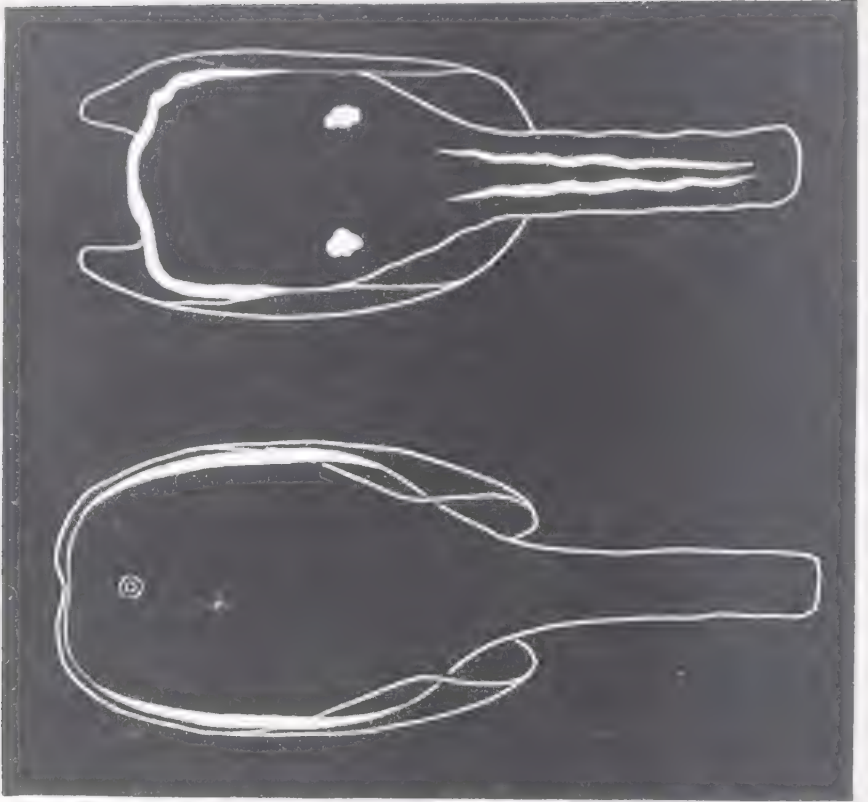


FIG. 80. Diagram of luminous areas of *Pholas dactylus* (above, after Panceri) and *Rocellaria grandis* (below, after Haneda).

streaks of light along the pallial lines, as shown in Fig. 80. The luminous secretion fills the mantle cavity and is occasionally spouted from the siphon. Haneda reported that the luciferin-luciferase reaction was positive.

CHAPTER IX

Cephalopoda

INTRODUCTION

Despite the external appearance, bilateral symmetry, and a high degree of activity, the cephalopoda are molluscs, forming a class of this great phylum. Far from leading sedentary lives, squid and octopi have developed the most modern methods of jet propulsion, and their eyes resemble those of vertebrates in form and perfection. It is not surprising that they should also include species with the most complicated lantern-like luminous organs with lenses, reflectors, and pigment screens. In one squid, light of different colors can be emitted. About 350 species of living squid are known, most of them with only a single pair of gills, the Dibranchiata. The other order, the Tetrabranchiata, contains numerous fossil species but a single living genus, the famed pearly nautilus.

All the luminous species are dibranchiate, mostly belonging in the suborder Decapoda, but one of the Vampyromorpha, *Vampyroteuthis* and possibly some Octopoda are luminous. Many squid are deep sea forms and it is among them that the number and complexity of luminous organs is greatest. A few species harbor luminous bacteria, a frequent but not inevitable association, and one remarkable deep sea squid, *Heteroteuthis dispar*, has developed the ability to extrude an abundant luminous secretion.

It is not surprising that students of the cephalopods should be intrigued with the variety of light organs. From time to time lists of luminous species have appeared. Hoyle (1908) recorded 30 luminous species of oegopsid and 3 species of myopsid squid, and Chun (1910) recorded 39 species of oegopsid squid. The best list is by Berry (1920),¹ a detailed and valuable compilation. Of 173 species of oegopsids, 99 or 57% are photogenic, while of 224 myopsids, 27 or 11% are photogenic. Only one or two of the 195 species of octopods are

¹ Summarized by Grimpe and Hoffmann in *Tabulae Biologicae*, Vol. 6, pp. 462-63, 1930.

luminous. The distribution of luminescence among families and genera of the cephalopoda, based largely on Berry's work, is indicated by italics in the following classification of J. Thiele.

Cephalopoda

Tetrabranchiata (Nautilidae, with single genus, *Nautilus*)

Dibranchiata

Vampyromorpha (*Vampyroteuthidae*, with single genus, *Vampyroteuthis*)

Decapoda (Squid, Cuttle-fish)

Oegopsida

Lycoteuthidae (*Lycoteuthis*, *Nematolampas*, *Lampadioteuthis*)

Enoploteuthidae (*Abralia*, *Abraliopsis*, *Watasenia*, *Ancistrochirus*,

Thelidioteuthis, *Pyroteuthis*, *Pterygioteuthis*, *Enoploteuthis*)

?*Octopodoteuthidae* (?*Octopodoteuthis*, *Octopodoteuthopsis*, *Taningia*,

Cuciteuthis)

Neoteuthidae (*Neoteuthis*) Photophores not mentioned

Onychoteuthidae (*Onychoteuthis*, *Cycloteuthis*, *Tetranychoteuthis*,

Onychia, *Chaunoteuthis*, *Anchistroteuthis*, *Moroteuthis*)

Gonatidae (*Gonatus*, *Gonatopsis*) No light organs

Psychroteuthidae (*Psychroteuthis*) Photophores not mentioned

Architeuthidae (*Architeuthis*) No light organs

Histioteuthidae (*Calliteuthis*, *Stigmatoteuthis*, *Meleagroteuthis*, *Histioteuthis*, *Histiochromius*, *Histiopsis*, *Histiotauma*)

Alluroteuthidae (*Alluroteuthis*)

Bathyteuthidae (*Bathyteuthis*, *Ctenopteryx*)

Brachyteuthidae (*Brachyteuthis*) Light organs unknown

Valyteuthidae (*Valyteuthis*)

Ommatostrephidae (*Hyaloteuthis*, *Ornithoteuthis*, *Eucleoteuthis*, and 7 other non-luminous genera)

Thysanoteuthidae (*Thysanoteuthis*, *Cirrobrachium*)

Chiroteuthidae (*Chiroteuthis*, *Chiroteuthoides*, *Chirosoma*, *Chiropsis*, ?*Echinoteuthis*, *Mastigoteuthis*, *Grimalkiteuthis*)

Joubiniteuthidae (*Joubiniteuthis*) Photophores not mentioned

Cranchiidae (*Leachia*, *Pyrogopsis*, *Liocranchia*, *Drechselia*, *Egea*,

Sandalops, *Cranchia*, *Liguriella*, *Phasmatopsis*, *Toxeuma*, *Anomal-*

ochranchia, *Bathothauma*, *Taonius*, *Verrilliteuthis*, *Megalocranchia*,

Leucocranchia, *Taonidium*, *Crystalloteuthis*, *Phasmatoteuthion*,

Galliteuthis, *Corynomma*, *Henseniteuthis*)

Myopsida

Loliginidae 17 genera, including *Alloteuthis*, *Doryteuthis*, and *Loligo*

Lepidoteuthidae (*Lepidoteuthis*)

?*Promachoteuthidae* (*Promachoteuthis*)

Sepiolidae (*Rossia*, *Heteroteuthis*, *Nectoteuthis*, *Iridoteuthis*, *Stoloteuthis*, *Euprymna*, *Sepioida*, *Inioteuthis*, *Rondeletiola* = *Rondeletia*, *Sepietta*,² *Sepiolina*)

Idiosepiidae (*Idiosepius*)

Spirulidae (*Spirula*)

² Luminous bacteria have been isolated from these genera, but have not been claimed as symbionts.

- Sepiidae (Sepia,² Sepiella,² Hemisepius)
- Sepiariidae (Sepioloidea, Sepiadarium)
- ?*Octopoda* (Octopus, Cuttle-fish)
- ?*Cirrata*
 - ?*Cirroteuthidae* (Stauroteuthis, Froekenina, Chunioteuthis, Cirroteuthis, ?*Cirrothauma*)
 - Opisthoteuthidae (Opisthoteuthis)
- ?*Incirrata*
 - ?*Bolitaenidae* (Japetella, Bolitaena with subgenus ?*Eledonella*)
 - Amphitretidae (Amphitretus)
 - Vitreledonellidae (Vitreledonella)
 - Octopodidae (20 genera)
 - Alloposidae (Alloposus)
 - Tremoctopodidae (Tremoctopus)
 - Ocythoidae (Ocythoe)
 - Argonautidae (Argonauta)

Many of the older zoologists had described the peculiar skin structures on cranchid (Grant, 1833) and enoploteuthid (Ruppell, 1844) squid, but the first observation of light from these luminous organs must be attributed to Verany (1851). In his great work, *Mollusques méditerranéens*, the volume on cephalopods contains a beautiful reproduction of *Histioteuthis Bonelliana* with its numerous large photophores and an account of a live animal caught at Nice in 1834. Although placed in a large bucket of sea water, the animal did not live long but nevertheless Verany observed that "during the night the opaline spots projected a phosphorescent splendor, making this mollusc one of the most brilliant productions of nature." *Histioteuthis ruppelli* is illustrated in Fig. 81.

The various expeditions of the last century greatly increased knowledge of deep sea squid. Verrill identified squid of the northeastern American coast but little attention was paid to luminescence. The *Challenger* expedition caught many deep sea squid, described by Hoyle who later (1902-12) studied the luminous organs of some forms. Joubin (1893-1924) in France added to the knowledge of light organ structure from material obtained by the *Travailleur* and *Talisman* and various expeditions of the Prince of Monaco. The *Valldivia* added more luminous species, and the leader of the expedition, Carl Chun (1903), studied the structure of eyes and light organs and identified all the Cephalopoda obtained. The final results (1910) were published in a profusely and beautifully illustrated monograph, which remains today a classic among zoological publications. Chun (1913) also studied the squid of the *Michael Sars* expedition and described the remarkable transparent deep sea genus, *Cirrothauma*.

Cephalopoda of the Humboldt-Plankton expedition were described

by Pfeffer (1912) and those of the Gulf of Naples of Naef (1921, 23) but few observations were made on luminescence. Robson (1948) studied the squid of the *Arcturus* expedition. Berry's (1920) important survey of light production also treats of the general biology of these animals. Many other authors have added to knowledge of certain species.

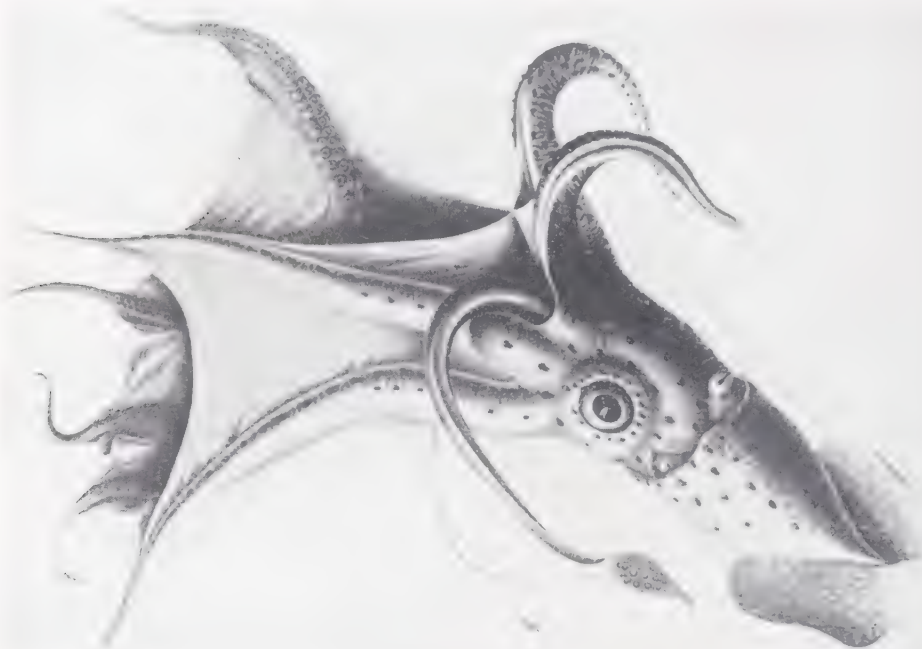


FIG. 81. *Histioteuthis ruppelli*. After Chun.

VAMPHYROMORPHA

The Vampyroteuthidae originally classed among the octopoda are known to possess photophores, but this family is so unusual that Pickford (1939) has suggested that it be placed in a new order, the Vampyromorpha, more closely allied to the Decapoda. Originally (Robson, 1932) a number of different genera (*Melanoteuthis* and others) and species were described, but Pickford, from a study of the existing material, has come to the conclusion that these are abnormalities due to poor preservation or stages in the development of one species, *Vampyroteuthis infernalis*. The original type specimen of *Melanoteuthis lucens*, described by Joubin is actually *Vampyroteuthis infernalis* and has the same types of photophores.

Vampyroteuthis infernalis is a bathypelagic form from upper deep waters of tropical and subtropical oceans, shown in Fig. 82. The mantle length is 50 to 80 mm, and the whole animal is jet black in

color except for the oral face of the web which is red brown. Three types of light organs are present. Near the apex of the body and just behind the base of each paddle-shaped fin there is a circular photophore (the fin light organ) which can be occluded by an eyelid. The

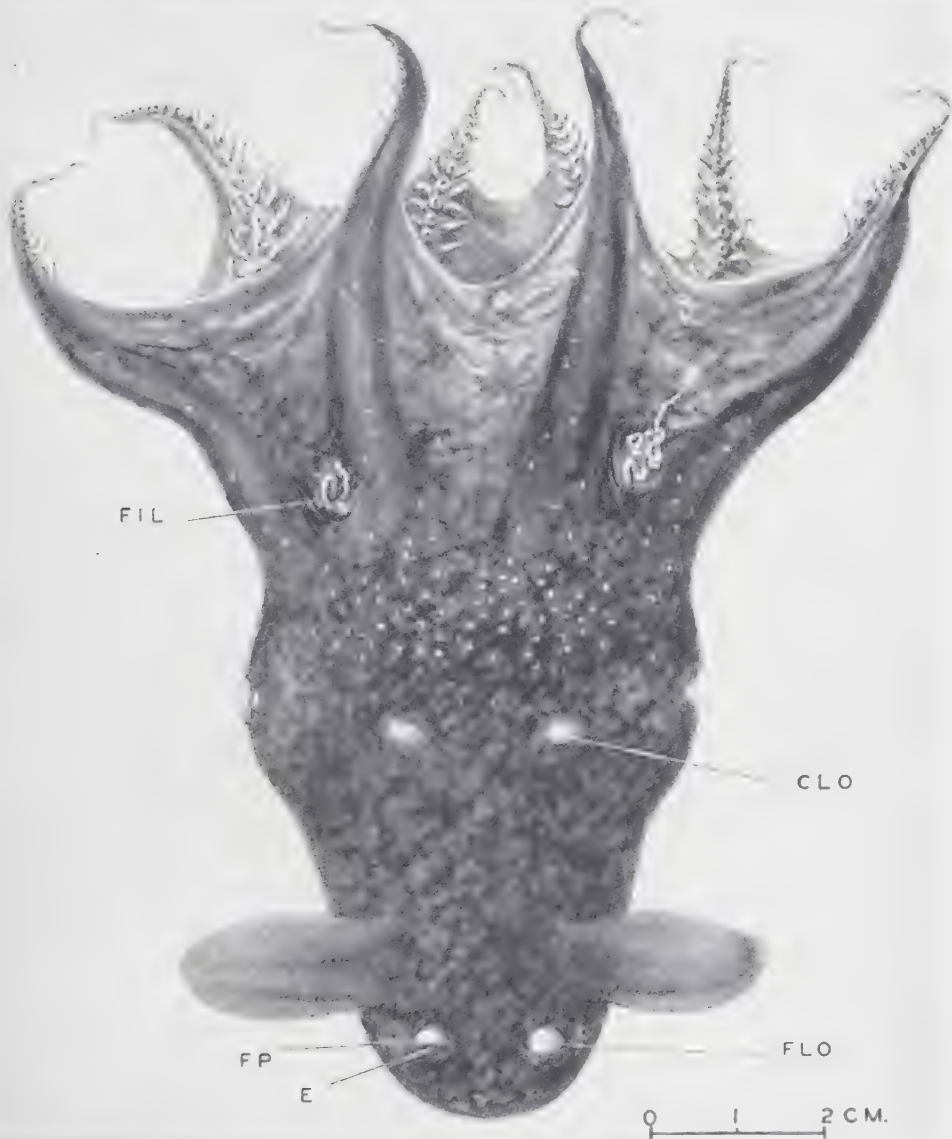


FIG. 82. *Vampyroteuthis infernalis*, drawn by Lisbeth Krause, supplied by G. E. Pickford from the Dana Reports.

second type is made up of clusters of luminous nodules on the back of the neck (the composite light organ), and the third type of minute simple organs which cover the whole external body surface except the oral face of the web. According to Pickford (1946, 49), these body

photophores are "A mere nodule of photogenic tissue immediately beneath the transparent epidermis backed by a connective tissue reflector and concentration of melanophores." The composite light organs lack the reflector. The fin light organs are larger and more elaborate, provided with a reflector and pigmented tapetum behind the photogenic mass as well as red and brown chromatophores in front. All are richly supplied with blood capillaries. The luminescence has never been observed.

DECAPODA

While a great deal of work has been carried out on classification and on the morphological and histological characteristics of preserved squid, less is known of the general biology, physiology, and biochemistry of living animals. For convenience of treatment, the true squid will be divided into three groups, depending on their method of light production.

- I Squid associated with luminous bacteria.
- II Squid producing an abundant luminous secretion.
- III Squid with well-developed photophores and intracellular luminescence. The I and II groups are all myopsid and the III group all oegopsid squid.

Squid Associated with Luminous Bacteria

General. Study of squid containing luminous bacteria begins with the work of Meyer (1906) who noticed glands in *Sepiola rondeletii* similar to those in *Heteroteuthis* and asked his friend, Werner Marchand, to determine if they produced light. Marchand found that the glands were indeed luminous, but contrary to the behavior of *Heteroteuthis*, no secretion was poured into the water at the slightest touch. The luminescence only occurred at the mouth of the gland and only after such violent stimulation as cutting away the mantle. Meyer also pointed out that not all specimens of *S. rondeletii* possessed the gland, that it was completely lacking in one-third to one-half of them. The absence of a gland could not be explained by sex differences or immaturity. He suggested that possibly two species occurred, one with and one without a light organ. He also found that in *Rossia macrosoma* there was a somewhat reduced light organ. Neither Marchand nor Weber realized that the light of *Sepiola* came from luminous bacteria.

Twenty years later Skowron (1926) investigated over thirty individuals of *Sepiola intermedia* by dissecting the surrounding muscles and uncovering the gland and found thirteen that gave no visible light

In the others the light was evident, and luminous bacteria were visible under the microscope. Skowron held that the bacteria of *Sepiola* were ordinary luminous bacteria which penetrated the organ at a late stage of development.

In myopsid squid there is no doubt that the light comes from bacteria and the discussion has centered around the question of symbiosis or chance association. In true bacterial symbiosis, the organisms must always be present, must be specific in characteristics and are presumably transmitted from generation to generation through the egg, as Pierantoni (1914, 18) and Buchner (1914, 21) have pointed out. The luminous glands of various species and the type of bacillus found in them have been extensively studied by three groups of active workers, one group in Italy led by Pierantoni and Zirpolo, another in Japan, led by Okada and Kishitani, and a third in Germany, represented by Meissner and Herfurth. Their conclusions will be discussed shortly.

Another early instance of a luminous squid in which light is now known to be due to bacteria has been recorded by Sasaki (1914), the lantern squid, *chochin-ika* (*Inioteuthis inioteuthis*, now known as *Sepiola birostrata*). He found the animal "discharging a faint cobaltish light from a great luminous organ which is situated in the mantle cavity—near the ink bag." The author saw this squid luminesce during his visit to Toyama Bay in 1916.

Most naturalists traveling in the West Indies have observed *Spirula* shells cast up on the beach. This unique shelled cephalopod has a well-developed non-glandular luminous organ at the posterior extremity of the mantle between the fins, an organ described from preserved material by a number of naturalists, without knowledge of its function and before Chun (1910) designated it a light organ. The luminescence has been observed by Schmidt (1922) who wrote: "On several occasions we were able to perceive that the small bead-like organ at the posterior end is a light organ. It emits a pale yellowish green light, which from the normal position of the animal in the water is directed upwards. In contrast to the light displayed by so many other marine organisms (Crustacea, etc.), which flares up and fades away again, the *Spirula*'s little lamp burns continuously. We have seen the lamp showing uninterruptedly for hours together." This description certainly agrees with bacterial light, which is also emitted continuously.

Although Okada (1927) described a definite luminous organ in *Spirula*, Herfurth (1936), who has made an extensive study of bacterial symbiosis in cephalopods, investigated the histology and came to the conclusion that the cells of the "Ringwulstes" might be photogenic although they contained only granules. No bodies that could be consid-

ered bacteria were present. The structure of the organ is shown in Fig. 83.

According to Pierantoni (1918) and Okada (1927), the common squid, *Loligo edulis*, has a pair of glands in the mantle cavity near the ink sac similar to those of *Sepiola*. Okada (1927) never observed light from the gland, and the common squid is not ordinarily thought of as luminous. However, Kishitani (1928) observed luminescence from



FIG. 83. Section of the light organ of *Spirula spirula* showing granules within the cells. After Herfurth.

these glands (shown in Fig. 84) of *Loligo edulis* from Japan and isolated from them a luminous bacterium, *Coccobacillus loligo*. He (1932) found that this bacillus also lives as a saprophyte on the outer surface of the mantle, together with ordinary luminous bacteria like *Pseudomonas phosphorescens*. Such facts illustrate the difficulty of deciding between symbiosis and parasitism.

Histology. The earlier workers considered that the light organ was an accessory nidamental gland having to do with formation of the jelly around the eggs. Although the gland has undoubtedly developed from the accessory nidamental gland it is actually distinct, and both Buchner (1930) and Pierantoni (1934) suggested the term "accessory

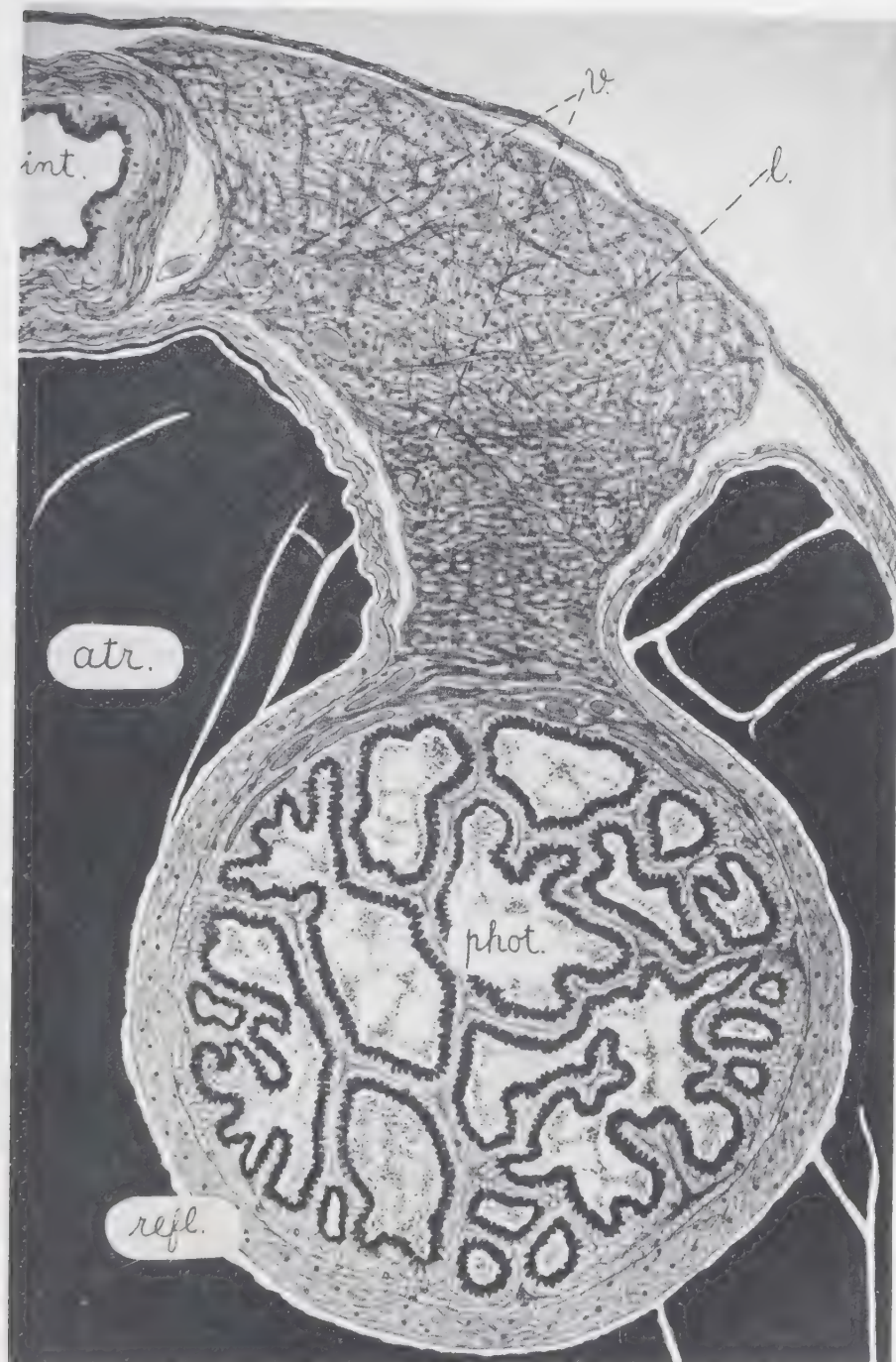


FIG. 84. Section of the light organ of *Loligo edulis*, showing light gland with bacteria. phot; reflector. refl; lens. l; ink sac, atr; blood vessels, v; rectum, int. After Kishitani.

gland." According to Herfurth (1936) accessory glands are present only in females of *Alloteuthis media*, *Spirula spirula*, *Rossia macrosoma*, *R. mastigophora* and *Sepietta oweniana*, while in *Sepiolo robusta*, *S. atlantica*, *S. ligulata*, *S. rondeletii* and *Sepiolo affinis*, they are present in both sexes. *Loligo forbesi* has a rudimentary accessory gland in the male. Usually the glands are paired but in *Rondeletia* the two form a median gland. Besides the list of genera with accessory glands described above, Robson (1926) has mentioned a *Loligo* from Indo-China with rectal glands observed to emit light, and Okada (1927) has added *Sepiella maindromii* and species of *Stoloteuthis*, *Iridoteuthis*, *Nectoteuthis*, and *Euprymna*.

Many workers have studied the fine structure of the accessory glands of various squid. Probably the best known are the various species of *Sepiolo*. Descriptions have been published by Meyer (1906), Dahlgren (1916), Pierantoni (1918), Okada (1927), Kishitani (1932), and Herfurth (1936). When the mantle is opened, the gland is visible as two white masses on each side of the median line near the anus. They lie against and are partially covered by the ink sac. Sections show that the specific cells of the organ are arranged in the form of sac-like glands from invagination of an epithelium. There are two papillae which mark the openings into the mantle cavity and also a lens (called Gallertkörper by Meyer), and a reflector, as shown in Fig. 85. The whole organ appears to have been designed for producing light.

Bacteriology. The principal workers on squid bacteria are Zirpolo (1917-24), Mortara (1922, 24), Meissner (1926), Kishitani (1928, 32), and Getzel (1934). Many have been able to grow luminous bacteria from myopid squid, but they differ in their views of the significance of the organisms. Zirpolo and Meissner have followed Pierantoni in believing that the bacteria are transmitted by way of the egg, while Mortara, Kishitani, and also Putoni (1925) have been skeptical. Kishitani pointed out that no one had been able to follow the cycle of a bacterial "inheritance," and Getzel was unable to isolate luminous bacteria from the accessory gland of *Sepia officinalis*, although he did grow color-producing bacteria which he considered symbiotic. -

Zirpolo (1918) first isolated *Bacillus pierantonii* from the accessory glands of *Sepiolo intermedia* and *Rondeletia minor* and *Bacillus sepiac* from *Sepia officinalis*. In a series of thirteen papers (1920-38) he reported on the effect of various agents on *B. pierantonii* and considered it a true symbiont. Meissner (1926) has also made an extended and detailed study, paying special attention to immune re-

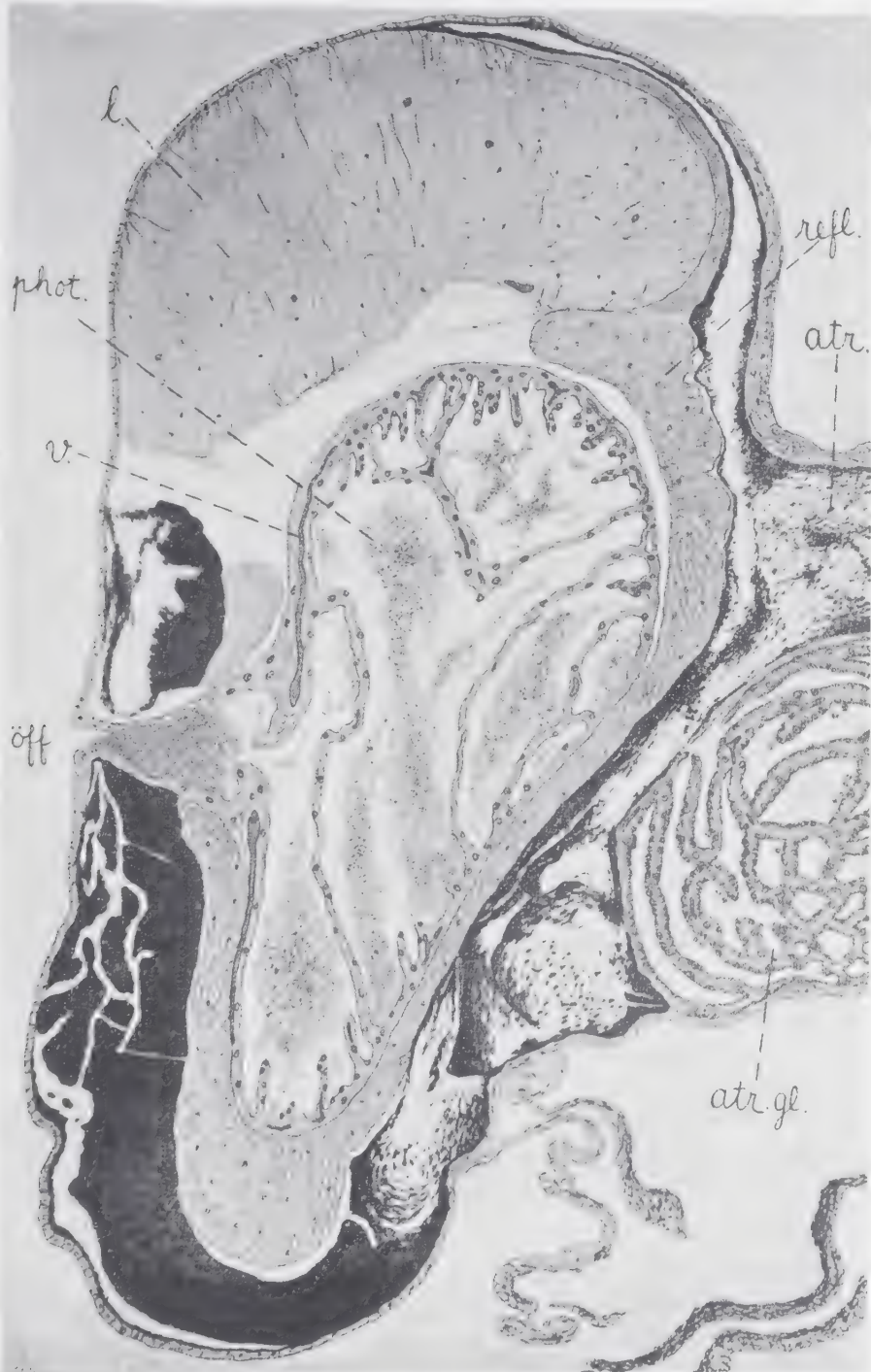


FIG. 85. Section of the light organ of *Sepiola birostrata*. Same lettering as in Fig. 84. Opening of the light gland, off; ink gland, atr. gl. After Kishitani.

actions and specificity. She isolated *Vibrio pierantonii* from *Sepiola intermedia*, *Coccobacillus pierantonii* from *Rondeletia minor*, *Vibrio sulla sepi*a (closely allied to *V. pierantonii*) and *Bacillus sulla sepi*a both from *Sepia officinalis*. These bacteria presented special growth characteristics and specific immune reactions which completely distinguished them from ordinary luminous bacteria living on the skin of marine animals. They were as different as the typhosus and the coli group, a circumstance which Meissner took to indicate a true symbiotic relation in the squid.

Another extensive study by Kishitani (1932) led to the same conclusions as regards special characteristics and immune reactions (agglutination) of bacteria from the accessory glands of Japanese squid. He isolated *Coccobacillus loligo* from *Loligo edulis*, *Pseudomonas empyrmyna* from *Empyrmyna morsei*, and *Micrococcus sepiola* from *Sepiola birostrata*. They each had special characteristics that indicated an adjustment to their particular host.

However, Kishitani also found these forms living as saprophytes on the outer surface of the mantle of their host squid and on the outer mantle surface of *Ommastrephes sloani pacificus*, a non-luminous squid. They were no less viable than ordinary luminous bacteria such as *Pseudomonas phosphorescens*, *P. toyamensis*, and *Coccobacillus ikien*sis. In the accessory nidamental gland of *Ommastrephes* there were other bacteria, some non-luminous and some luminous, but quite different from the symbiotic luminous forms. Although referred to as symbiotic in his papers, Kishitani held that these bacteria were not passed from generation to generation through the egg but entered the light organ of the host from the outside. He also concluded that no bacteria were present in the closed organs of such oegopsid squid as *Watasenia scintillans*, *Abrabia japonica* [described by M. Ishikawa (1929) from the Sea of Japan], *Enoploteuthis chunii*, and *Chiroteuthis imperator*, although the light organs of these forms do contain rod-shaped or spindle-shaped bodies in all cases except *Chiroteuthis*, where they are small granules.

Although the secretion of accessory glands of myopsid squid does cover the eggs with luminous bacteria at the time of laying, there is no evidence that bacteria penetrate the egg as Pierantoni suggested. Herfurth (1936) studied the egg laying in *Sepia officinalis* and found no bacteria in the perivillelline fluid in which the embryo is suspended nor in the epithelial or subepithelial tissues of early embryos. Hatching embryos are not covered with bacteria, and embryos with mantles 1.8 cm long, when the Anlagen of the accessory glands are present, contain no bacteria. Only in those embryos with mantles 2.5 to 3.5

cm long could Herfurth discover bacteria in the accessory glands, and he concluded that the bacteria entered from the outside. It is thus evident that the case for true symbiosis of luminous bacteria and squid is not too strong.

Squid Producing a Luminous Secretion, Heteroteuthis dispar

Considerable controversy has arisen in regard to the self-luminosity of *Heteroteuthis dispar*, a deep sea squid, occasionally caught along the coasts of southern Italy, particularly in the Strait of Messina where whirling currents bring many bathypelagic organisms to surface waters. There is a passage in Aristotle which may refer to this squid, which was named by Ruppell. Modern study begins with Meyer (1906, 08) who learned of the light secretion of the gland from Lo Bianco in Naples and studied the morphology and histology of the light organ. *Heteroteuthis* has been seen in action by numerous investigators including the author. There is usually no luminescence from the undisturbed animal or occasionally there may be a faint light but whenever touched, the secretion is spurted through the funnel in masses which contain many points or threads of bright light. The yellowish luminescence of the points dissipates in the sea water and fades out after some time.

When Pierantoni (1918) found luminous bacteria in *Sepiola* and *Rondeletia*, he suggested that *Heteroteuthis* might also contain them and in 1924, from a study of the large luminous gland, confirmed his earlier opinion. Mortara (1922, 24) was unable to agree and held that the organ had a purely glandular function since no luminous bacteria could be isolated in cultures from the light gland, although many grew from the skin.

Under the microscope the points of light in the secretion are observed to be clumps of yellowish granules with a few large colorless ones. Often the yellow granules are distorted to a reniform shape. These granules are the symbiotic bacteria of Pierantoni, but they are non-mobile and very different from ordinary luminous bacteria as will be pointed out in the section on biochemistry. The evidence is against the bacterial theory.

Morphology and Histology. The morphology and histology of the gland has been studied by Meyer (1906, 08), Pierantoni (1924), and Herfurth (1936). It is present in both male and female, just posterior to the anus and more or less surrounded by a rather small ink sac. These squid, even though they live in total darkness do also eject a small amount of ink when disturbed. The gland has structures called lenses, photogenic cells, a reservoir with two discharge openings indi-

cating its paired origin, and muscles to squeeze out the secretion. These parts are shown diagrammatically in Fig. 86. The cell structure is quite different from that of the nidamental glands and accessory nidamental glands which are present only in the female.

Biochemistry. During a trip with Dr. S. Skowron to Messina in 1925, the author was able to carry out a number of experiments on the secretion of *Heteroteuthis*. Some of these have been reported by



FIG. 86. Section of the light organ of *Heteroteuthis dispar*, showing granules, n, in compartments of the gland; reflector, rl; lens, l; ink sac, tn; blood vessel, v, v'; epithelium, ep. After Pierantoni.

Skowron (1926). It was established that considerable oxygen is necessary for luminescence. The secretion on a microscope slide under a cover slip soon became dark and luminesced only at the edge in contact with air. When the cover slip was removed, the whole secretion luminesced. The necessity of oxygen was also checked by the addition of sodium hydrosulfite, which quickly absorbs oxygen; the light then disappeared, to return on shaking with air. The light organs could be dried over CaCl_2 and would emit light when fresh water was added. There was no inhibition of the luminescence by daylight and no marked fluorescence of the secretion in ultraviolet light.

The author was unable to demonstrate a luciferin-luciferase reac-

tion, despite many attempts and the preparation of the luciferin solutions by heating to low temperatures to guard against possible destruction on boiling. There was also no light when Cypridina luciferin was mixed with Heteroteuthis extracts which should have contained luciferase or on mixing Cypridina luciferase with Heteroteuthis "luciferin." It is by a comparison of a sea water suspension of luminous bacteria from *Sepiola intermedia* and the Heteroteuthis secretion in sea water that interesting differences appear, as pointed out by Skowron (1926). The bacterial light of *Sepiola* lasts over twenty-four hours while the Heteroteuthis light disappears in less than three. When two parts of sea water are added to the luminous bacterial suspension the light disappears in about seven minutes, whereas it disappears immediately in the case of Heteroteuthis secretion with no increase in brightness. When heated the light of the *Sepiola* bacteria disappears at about 40°C., that of Heteroteuthis at about 34°C. Both luminescences return on cooling.

It can always be argued that the granules of Heteroteuthis are bacteria so modified by intracellular existence that their properties are completely changed, an argument that can be and has been applied to the granules characteristic of all luminous organisms. It is known that fat-solvent narcotics extinguish the light without a preliminary flash, but the effect of cytolytic substances like saponin has not been tested. It would be most important to determine if their oxygen consumption per milligram is comparable with that of bacteria. Lack of material has prevented attempts to extract luminous substances by various organic solvents, an important line of future study.

Use of Light. It is sometimes said that the luminous secretion of deep sea Heteroteuthis has replaced the ink of surface dwellers. This black fluid forms a poisonous smoke screen which confounds an enemy while the squid makes a getaway. Although it is not true that the luminous secretion has completely replaced the ink, it may serve a similar purpose—to draw the attention of the enemy to a mass of lighting material, while the squid moves off in another direction. Such an explanation seems reasonable, although no attempts to confirm it have been carried out.

Squid with Photophores and Intracellular Luminescence

Since the observation of light emission from the photophores of *Histioteuthis bonelliana* by Verany (1851), a few additional forms have been seen to luminesce. However, except for squid living near shore at the surface, these occasions have been few and far between. The most famous case of squid luminescence is the description by Chun

1903) of *Thaumatolampas* (*Lycoteuthis*) *diadema*, caught in the Indian Ocean on the "Valdivia" expedition at a depth of 3,000 meters. Two specimens were brought up alive and were kept in iced sea water long enough to study and to photograph while alive. The animal shown in Fig. 90 has five light organs on each eyeball, eight on the under side of the body, and four on a pair of arms. Chun said: "One would think that the body was adorned with a diadem of brilliant gems. The middle organs of the eyes shone with ultramarine blue, the lateral ones with a pearly sheen. Those towards the front of the lower surface of the body gave out a ruby-red light, while those be-

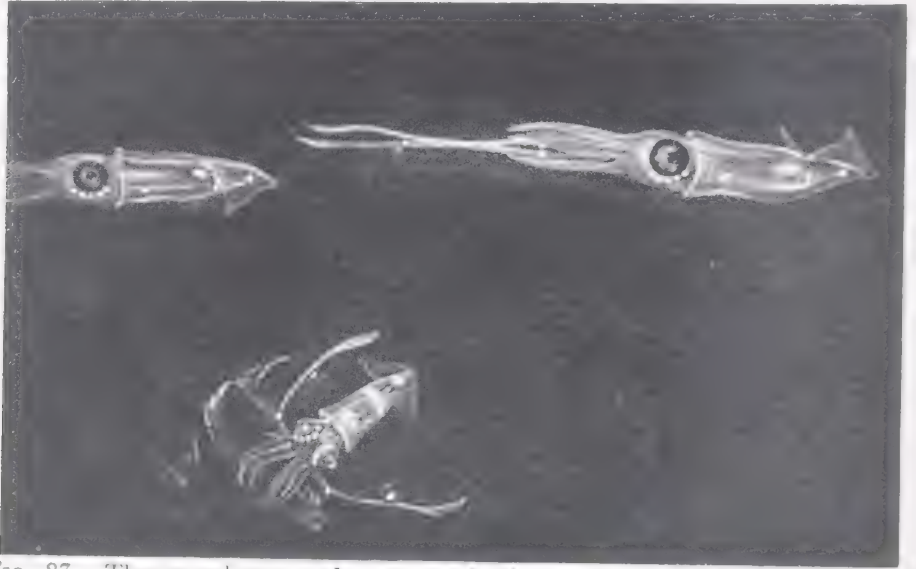


Fig. 87. *Thaumatolampas* (*Lycoteuthis*) *diadema* as it might look in the deep sea. After Dahlgren, from a drawing by Bruce Horsfall.

hind were snow-white or pearly, except the median one, which was sky-blue. It was indeed a glorious spectacle." The squid with its luminous organs aglow is illustrated in Fig. 87.

A close rival of *Lycoteuthis diadema* is a remarkable member of the same family, *Nematolampas regalis*, described by Berry (1913) from Sunday Island, one of the Kermadec group. This species, 57 mm body length, has at least ninety light organs in all, most of them large. There are five on each eyeball, ten within the pallial chamber, and seventy on arms or tentacles, as diagrammed in Fig. 88. Unfortunately the animal has never been seen alive.

Another deep sea squid was observed alive and described by Watase (1905) in a Japanese paper, the "hotaru-ika" or fire-fly squid of Japan, *Watasenia scintillans*, originally called *Aburatsubo scintillans*. This squid has a mantle length of some 5 cm. There are three black spots,

actually luminous organs, on the tips of each of the ventral pair of arms, five light organs on the eyeball, and many small light organs scattered over the body. After pointing out that the three black spots on the ventral arm tips produce light, Watasé said:³ "Again there are hundreds of other small spots all over the body. . . . When seen in daylight they appear to be small black spots, but in the night all these spots shine with a brilliant light like that of the stars in heaven. . . . When these spots (while the hotaru-ika is alive) are viewed under the microscope, they are very interesting. When the animal is about to produce the light, the membranes [chromatophores] covering the spots will concentrate and remove themselves, thus opening a way for

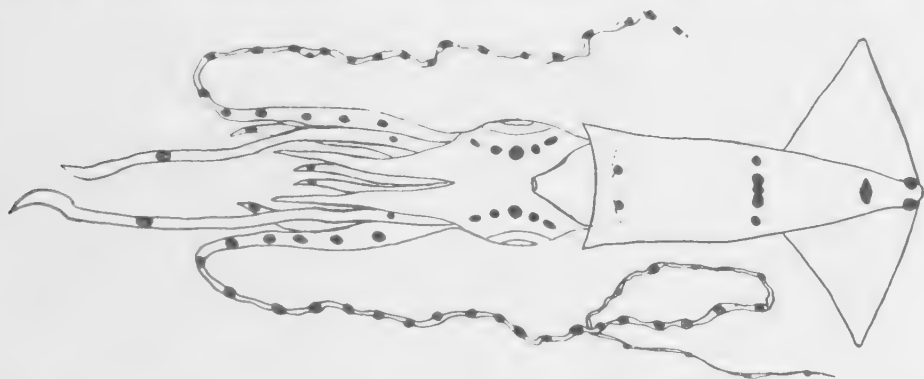


FIG. 88. Diagram of the light organs of *Nematolampas regalis*. After Berry.

the light. The light is so brilliant that it seems like a sunbeam shot through a tiny hole in a window curtain. Again when the hotaru-ika wishes to shut off the light, the membranes will expand and cover the spots. . . . " The animal is shown in Fig. 89.

Since then the hotaru-ika has been observed alive many times and studied by a host of workers—Sasaki (1912, 13, 14), C. Ishikawa (1913), Shoji (1917), Harvey (1917), Shima (1927), Hayashi (1927), Kishitani (1928), Okada, Takagi, and Sugino (1933), Takagi (1933), Yamada (1937), and Hasama (1941). More is known of this squid than of any other, because, although a deep sea form, it comes to the surface in Toyama Bay⁴ each year during late April, May, and June to breed. At this time females predominate. The towns of Uodzu and Namericawa are best for collecting, as the fishermen obtain them in great numbers (1,000 tons total catch each year) in nets and use them as fertilizer on the soil. Sasaki has dealt particularly with commercial uses. The author has seen a net while still in the water filled with

³ A translation by S. Matsushita, taken from Berry (1920, p. 163).

⁴ Another new species of a related squid, *Abralia japonica* has recently been described by M. Ishikawa (1929), also from the sea of Japan (Toyama Bay).

the animals, their arm organs flashing in bursts of a bluish light that was extraordinarily bright. The animal is well named—fire-fly squid—and gives a startling display of fire works, but it is very delicate and cannot be kept in aquaria for any length of time.

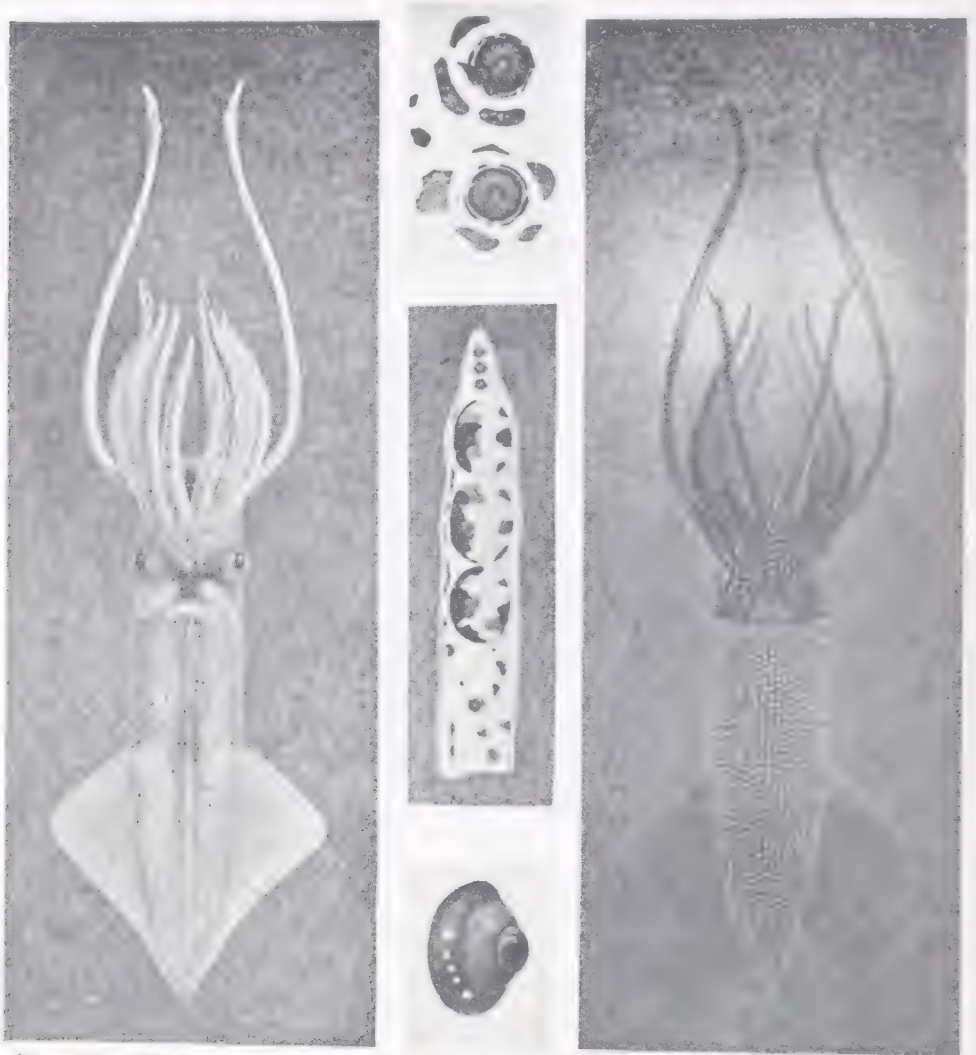


FIG. 89. *Watasenia scintillans*, by day (left) and at night (right), with enlarged views of the three types of light organs, cutaneous (above), arm tip (middle), and eyeball (below). After Sasaki.

A similar migration from the depths for spawning has been recorded by Berry (1926) in *Abrabia veranyi*, previously known only from the Mediterranean and first observed by Ruppell (1844) at Messina. *Abrabia* does not appear in the enormous numbers characteristic of *Watasenia*, but can be obtained during July, August, and September in sheltered coves in the harbor of Funchal, Madeira. The five eye

organs have been described as particularly brilliant, of an ultramarine blue. The arms, body, and head are also covered with lights, especially on the ventral side.

Other deep sea squid with unusual combinations of light organs described by Chun but never seen alive should be mentioned. *Abrolipsis morisii*, very similar to *Watesenia scintillans*; *Pyroteuthis margaritifera* with six organs on each eyeball and ten on the under side of the body; *Enoploteuthis leptura* with an arc of ten large organs in each eyeball and many others scattered over the eyeball, arms, and ventral side of body; *Pterygioteuthis giardi*, with thirteen organs irregularly arranged on each eyeball and seven on the ventral surface of the body; *Calliteuthis giardi* with many large organs on the under side of the body and tentacles, as in the case of *Histioteuthis Ruppelli*. Finally the most bizarre of deep sea squid, animals like *Toxeuma belone* and *Bathothauma lyromma* with eyes at the end of stalks, like the optical system of a range-finder, and with light organs on the eyeballs. Some of these various types are illustrated in Figs. 90 and 93.

An extraordinary characteristic of the genera *Histioteuthis* and *Calliteuthis* is the enormous development of the left eye, which is much larger than the right. The right eye has a well-developed circlet of photophores, but in the giant left eye the photophores are far apart, distorted, and in some cases reduced to a rudiment. No explanation of this unusual asymmetry in relation to habits or behavior has been offered.

The distribution of true photophores in squid has been fully treated by Berry (1920). It is sufficient to point out that they occur on every conceivable region, head, eyeball, around eye openings, mantle, arms, tentacles, funnel, fins, and in the intrapallial cavity. Each species has a slightly different arrangement, and in the various families the distribution may be quite different. The most usual and at the same time a most unexpected place is the eyeball. There must be a good reason for this position, for not only squid but euphausiids and deep sea fish have luminous organs near their eyes. Such a position might be a proper one for seeing if the light organ were a searchlight. In addition to those on body and arms, *Histioteuthis* has a circle of photophores not on the eyeball but around it and Joubin (1893) has remarked that these organs seem to be well adapted to illuminate the dark places where the cephalopod lives and perhaps to attract to its neighborhood animals on which it preys. Extended observation of living deep sea squid may give a clue to the use of so complicated a set of light organs, but at present the reason for eyeball photophores is certainly problematical.

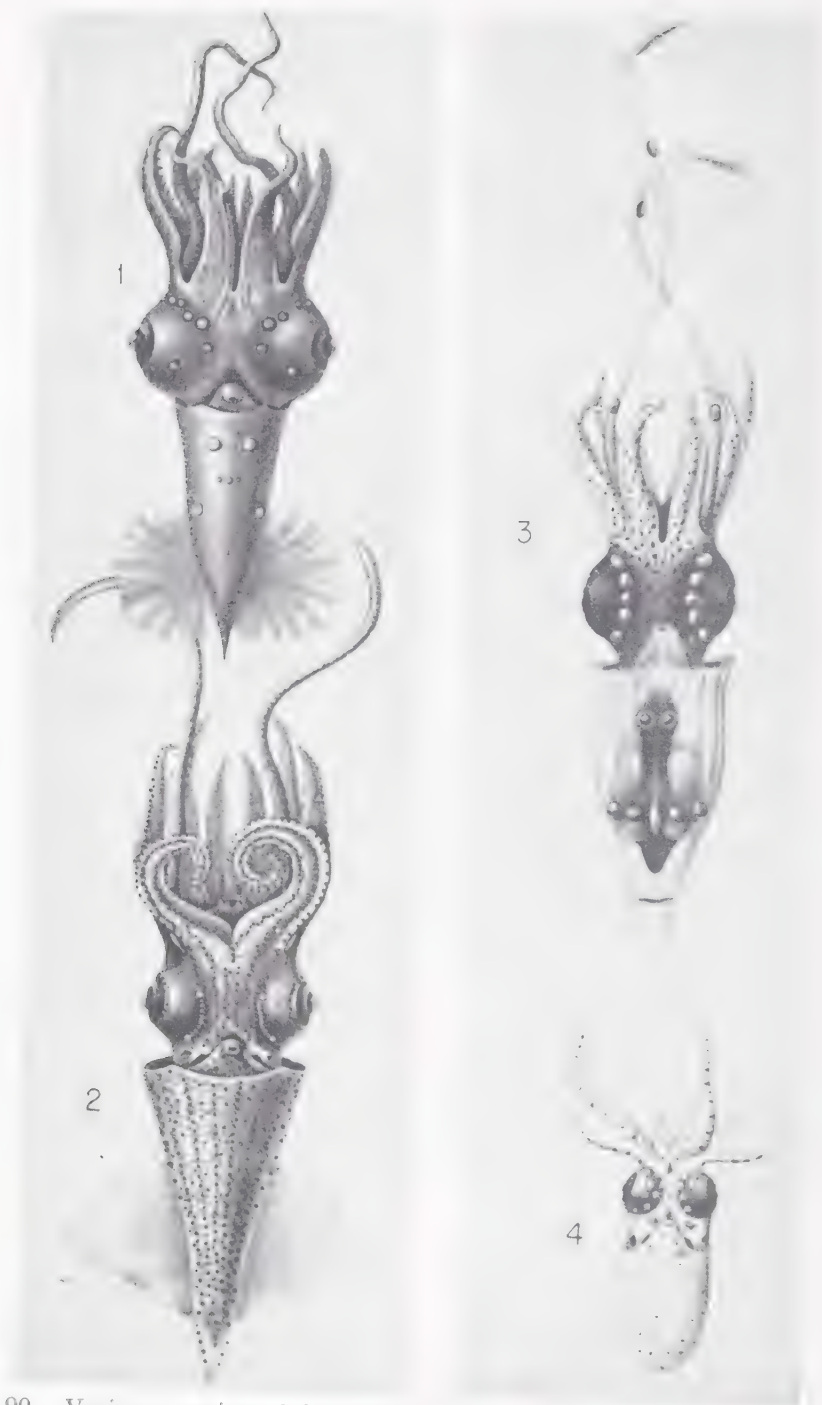


FIG. 90. Various species of luminous squid. 1, *Pyroteuthis margaritifera*; 2, *Euproteuthis leptura*; 3, *Thaumatomolampas* (*Lycoteuthis*) *diadema*; 4, *Calliteuthis* young.

The embryology and development of the light organs of squid is an almost untouched field. Even those deep sea squid which breed at the surface presumably return to the depths. However, a number of young have been caught from time to time and described by Chun and other workers. Grimpe and Hoffmann (1921) have dealt with post-embryonic stages of *Histioteuthis*, particularly the light organs around the eye and the time of appearance of the web between the arms, but Degner (1923) is not in agreement with all the findings. Until deep sea squid can be raised in captivity the stages in development of the light organs will remain largely unknown.

Histology. Although Hoyle's monograph on Cephalopoda of the *Challenger* expedition appeared in 1886, no attention was paid to luminescence or to the structure of the large number of "papillae" which were conspicuous in Hoyle's figure of *Histiopsis*. Since that time the histology of most photophores has become well known. The variety is so great that only a general picture can be given. Berry (1920) has classified the different types which range from "the lump of photogenic tissue which forms the proximal photophore in the tentacle of *Lycoteuthis*, through almost innumerable intermediate types to the astonishingly complex, bull's eye lanterns of *Abraliopsis* and the mirrored search lights of the *Histioteuthidae*." Sometimes there are accessory photophores or double organs, as in the subocular structures of certain cranchids and a number of those of *Lycoteuthis diadema*. Often a single animal will have two, three, or many quite different types of photophores.

The first histological study was made by Joubin (1893, 94) on *Histioteuthis Ruppelli* from Nice. The study was of sufficient interest to warrant a popular article on the subject in *La Nature* by Coupin (1893), who noted that the microtome had replaced the hand lens in study of animals. From arm tip to arm tip *Histioteuthis* measures 1 meter. Its light organs are nearly two hundred in number, of ellipsoid shape, 7 mm long and 6 mm wide. Joubin's figure of one of the body organs shows a mirror, with chromatophores behind the mirror and also behind the reflector of the photogenic apparatus proper, which is made up of a luminous cell layer, a transparent cone and two lenses, biconvex and concavo-convex. A nerve enters through the reflector.

Grimpe and Hoffmann (1921) have made a particularly careful study of the end light organ on the sixth arm of *Histioteuthis bonelliana*. The end organ has a structure like the arm organs of *Abraliopsis* or *Watasenia*, very different from the lamp-like organs of the body. The slit-like opening of the end organ, called "Streifenleuchtor"

gane" because of their striated character, can be closed by chromatophores, just as the lamp-like organs can be screened by a single chromatophore in the optic axis. The light of the end organ is not focused but diffuse, since there is no lens or mirror. One of the marked structures of both organs is a nerve cell layer in the periphery



FIG. 91. Section of the anal light organ of *Pyroteuthis margaritifera*, showing pigment, pg; reflector, rft; photogenic cells, pgc; and lenses, l, l'. After Mortara.

of the photogenic region. Grimpe and Hoffmann have suggested that this layer may have a sensory function and the organs a double use, not only to produce light but also to perceive it. In this case the ganglion cell layer would act as a retina and the chromatophores as a diaphragm. The suggestion is interesting but does not seem likely as the nerve structures could be involved in exciting the luminescence.

In addition to later studies of Joubin (1905), the histology of other

European forms has been worked out by Hoyle (1902-12), Chun (1903, 10), Tippmar (1913), Vivanti (1914), Mortara (1917, 21, 22), and Okada (1927). The histological picture in the Japanese *Watasenia scintillans* is very similar to that of *Abraliopsis morisii* described

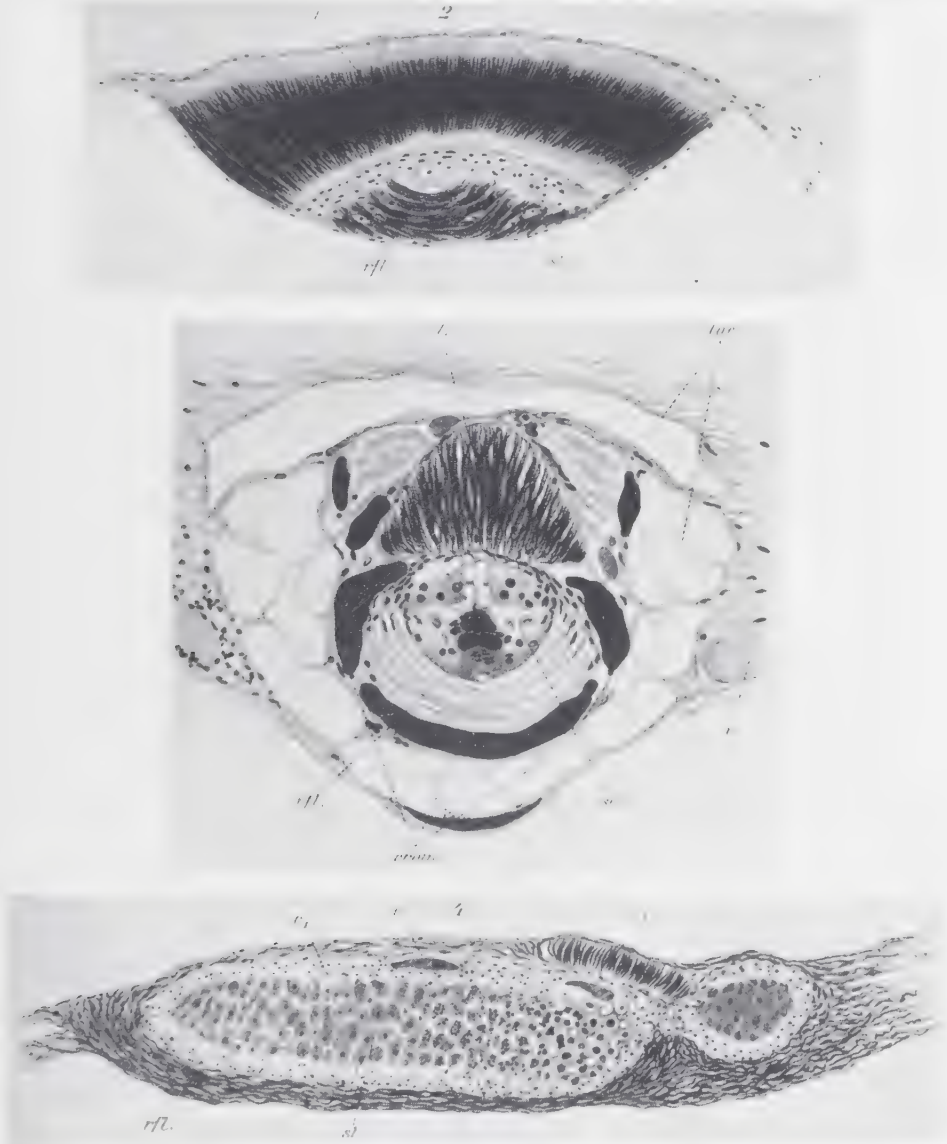


FIG. 92. Section of the light organs of *Abralia veranyi*. Above, ocular organ No. 2. Middle, cutaneous organ. Below, ocular organ No. 5. After Mortara.

by Chun. Sections of the various types of organs have been described by Dahlgren (1916), Shima (1927), Hayashi (1927), Kishitani (1928), and Tagaki (1933). The investigations are of particular interest, since Shima (1927) had claimed that rod-shaped bodies in the

cells are luminous bacteria, an interpretation not shared by other investigators except Hasama (1941) who was undecided.

Shima (1927) described his "bacteria" as gram negative forms in fresh smears. The length was 1 to 3 μ and the width 0.5 μ . He claimed to have grown them as facultative anaerobes on 3% salt gelatin. Sometimes luminescent colonies developed and sometimes



FIG. 93. *Leachia valdiviae* (left); *Cranchia scabra* (middle) and *Toxeuma belone* (right). After Chun.

not. Hayashi, Kishitani, and Tayaki have all observed these bodies, but they reject the bacterial interpretation, and Kishitani was unable to grow luminous bacteria from *Watasenia*. Tagaki described minute granular or rod-shaped mitochondria in addition to the larger rods. Hayashi considered that the large rods played an important part in luminescence, but Kishitani was not certain whether they were photo-

genic or reflector in function. The microchemical tests of Okada, Takagi, and Sugino (1933), described under biochemistry, definitely dispose of the idea that they are microorganisms and suggest protein crystalloids. The cells in which the rods are found are richly supplied with blood capillaries but nerve fibers have not been demonstrated.

Among the twenty-two light organs of *Lycoteuthis diadema*, Chun found ten different types. Sections of some of the complicated light organs of *Pterygioteuthis* (*Pyroteuthis*) *margaritifera* (Hoyle, 1902; Chun, 1903; Mortara, 1917, 21), *Chiroteuthis* (*Abralia*) *verangi* (Chun, 1903; Mortara, 1917) are reproduced in Figs. 91 and 92. Although Joubin (1893) once described a modification of chromato-



FIG. 94. Section of a light organ of *Leachia eschscholtzia*. REFL., reflector. EP, epithelial layer; IN, last trace of lumen at the point where the epithelium invaginated to form the luminous cell mass. After Dahlgren.

phores of *Chiroteuthis Bomplandi* as a thermoscopic eye to detect infrared, the interpretation is questionable.

The cranchid squid shown in Fig. 93, are most unusual in appearance and have particularly primitive light organs. In *Leachia* (Joubin, 1905; Chun, 1910) they are little more than invaginated epithelial cells which have developed the ability to produce light. There is also a trace of reflector as shown in Fig. 94.

Hoyle (1912) has described the luminous organ on the stalked eye of *Bathothauma lyromma* as consisting of three parts: (1) a kind of cup of oblique shape, made up of scales like the reflector of other photophores; (2) a granular mass of tissue without definite cell boundaries in the bottom of the cup, apparently the photogenic cells; and (3) a mass of elongated fiber-like cells with their long axes parallel to the long axis of the photophore but bent at an angle near the outer surface. There is no clear line of demarcation between the elongate cells and those of the photogenic mass.

Physiology. The rarity of squid with photogenic organs has made physiological work difficult, except in the case of *Watasenia scintillans*. The author (1917) and Shoji (1919) have studied several aspects of their physiology. The animals never live well when caught, an effect due largely to the low osmotic pressure of surface water of Toyama Bay, which is considerably more dilute than that of the depths. By keeping the animals in oxygenated (by pure O_2) sea water from below a depth of 5 meters they lived for twenty-four hours, whereas without oxygen in surface water they die in six hours.

The author noted that the excised arm organs, whose light is bluish quickly fatigue and lose their ability to luminesce on mechanical or electrical stimulation, either direct, or indirect through the nerve ganglia. Even when not stimulated, the ability of an excised organ to luminesce is soon lost. After death these organs become covered with pigment, but Shoji proved that the pigment was not merely masking the light. Even when the pigment was removed the arm organs would not luminesce when stimulated. The eye organs are difficult to stimulate, but they otherwise behave like the skin organs.

The skin organs, whose light is yellowish, can be readily investigated by removing the mantle and keeping it bathed with fresh sea water. On electrical stimulation a bright light appears from each organ. Shoji studied the effect of ether, chloroform, and alcohol vapor on these organs in a gas chamber and noted the initial excitation by the narcotic followed by disappearance of the light (narcosis), with subsequent recovery when the narcotic was removed.

The usual effect of temperature on luminescence was also observed, a decreased intensity at low and high temperatures. The light disappears at $44.5^\circ C$ after one minute but reappears on cooling. The luminescence of organs on the mantle is not affected by considerable changes in osmotic pressure and, according to Shoji's table, persists for an hour in distilled water. Hypotonic but not hypertonic solutions stimulate the light emission. Alteration of various ion concentrations in the sea water was studied by Shoji, but these experiments should be repeated with more carefully controlled conditions.

The mode of stimulation to light emission offers an interesting problem in *Watasenia* and probably in other squid. The light of the arm organs is periodic, like the fire fly, but with longer and more variable emission periods, occasionally lasting thirty seconds. Each of the three organs in an arm can emit light separately or the three can luminesce together. Although the brachial nerve lies next to the arm organs, Hayashi and others have been unable to demonstrate any distribution of nerve fibers in the light organ itself. Control of the

light appears to be as Watasé originally stated, through expansion and contraction of the covering layer of chromatophores. The skin organs are also surrounded by chromatophores which can be seen to contract and become globular when light is emitted and then to expand and cover the organ thus cutting off the light. This method of control implies that light is emitted continuously within the organ, a point which has not yet been determined. It is possible that chromatophore contraction and nerve stimulation of the photogenic cells may occur simultaneously. The eye organ is apparently not controlled by chromatophores but further observation is needed.

An interesting experiment not yet tried has to do with the possible hormone control of luminescence in squid. Although the chromatophores of cephalopods are under nerve control, it is possible that light emission might be affected by adrenaline and other hormones as is the case in certain fish.

Hasama (1941) has described action potentials accompanying luminescence of *Watasenia*. The luminous organs are weakly electronegative to non-luminous skin regions but during light emission the negative potential increases rapidly, and when the luminescence ceases returns slowly to its original value.

Biochemistry. Very few chemical studies have been made on squid with photophores and these have all been carried out on *Watasenia scintillans*. The author (1917) noted that if fresh arm photophores were ground in a mortar with sea water the light disappeared quickly. If fresh arm organs were cut off and placed in sea water for some thirty minutes and then ground, no light whatever would appear. Using fresh arm photophores, it was not possible to demonstrate luciferin and luciferase with extracts prepared in the usual way.

Shoji (1919) proved that oxygen is necessary for luminescence. He used the skin organs of the mantle placed in a gas chamber. When hydrogen (or CO_2) was passed over the skin organs, their luminescence disappeared to return again when air was readmitted.

Various extraction techniques have not been used in an attempt to isolate luminous material, but the rod-shaped or spindle-shaped bodies in the light organs of *Watasenia* have been studied microchemically by Okada, Takagi, and Sugino (1934). In fixed material containing formalin these bodies dissolve, important evidence against their bacterial nature and a probable reason for the fact that they were overlooked in the histological studies of earlier workers. Solubility in dilute 1% HCl and glacial acetic acid and ammonia also distinguishes them from bacteria. They are insoluble in distilled water, alcohol and such fat solvents as ether and chloroform, which do not appear to

change them in any way. No birefringence could be observed. They do not stain in fat stains like Nile blue or Sudan III. Their protein nature is attested by complete digestion in trypsin and by a positive reaction to protein color tests (xanthoproteic, Millon, and biuret).

OCTOPODA

It is highly probable that no true octopus is luminous, although a few references occur. Among these, the most interesting is a statement of Darwin from the voyage of the *Beagle*: "I was much interested, on several occasions, by watching the habits of an Octopus, or cuttle-fish. . . . I observed that one which I kept in the cabin was slightly phosphorescent in the dark." It is possible that Darwin observed luminous bacteria. His statement implies that the octopus was alive when observed, but if dead the phosphorescence was certain to have been due to luminous bacteria, which grow abundantly on dead squid and octopi. Bacteria may account for the luminous octopus mentioned by Eydoux and Souleyet in "*Voyage sur la Bonite*."⁵

A more recent statement comes from Gardiner and Cooper (1907). While dredging between Ceylon and Mauritius they caught at 750 fathoms "a curious gelatinous cuttle-fish (*Eledonella*) set with minute black specs, each giving a tiny spark of rather blue phosphorescent light." Possibly the word "phosphorescent" was used when "iridescent" was meant.

Not enough is known of the deep sea species, *Cirrothauma murrayi*, to call it luminous. Chun (1913) described the animal as transparent and fragile as a ctenophore, with rudimentary eyes and considered it the only blind cephalopod. On the arms, the gelatinous sucker stalks contained structures which might be light organs, but Chun concluded that only observation of the living animal could definitely settle the question.

⁵ *Zoologie*, 2, 14, 1852, quoted from Robson (1932).

CHAPTER X

Crustacea

CLASSIFICATION

Crustacea belong to the great phylum, Arthropoda, containing more than 75% of the total number of species of the animal kingdom. The Arthropoda may be conveniently subdivided as follows:

- Crustacea* (crustaceans), 25,000 species
 - Entomostraca* (brachiopods, copepods, ostracods, barnacles)
 - Malacostraca* (shrimp, crabs, lobsters)
- Arachnoidea* or *Chelicerata*, 36,000 species
 - Xiphosura (Limulus or king crabs)
 - Pycnogonida* (sea spiders)
 - ?*Arachnida* (spiders, scorpions, mites)
- Myriapoda*, 2,000 species
 - Pauropoda
 - Diplopoda* (millipedes)
 - Chilopoda* (centipedes)
 - Symphyla
- Insecta* (insects), 577,000 species

Groups in italics contain luminous species, and it will be observed that luminescence is widespread among the Arthropoda. The Chilopoda (centipedes) are sometimes placed near the Insecta and called Opisthogoneata, as contrasted with the Pauropoda, millipedes (Diplopoda) and Symphyla, which are collectively called Progoneata, while the Crustacea are given a superclass rank equivalent to Progoneata and Opisthogoneata. King crabs and spiders are definitely related.

Among the orders of Crustacea, five contain known self-luminous species, the Copepoda, Ostracoda, Mysidacea, Euphasiacea (Schizopoda), and Decapoda. Two additional orders, the Amphipoda and Isopoda, occasionally contain forms (sand fleas and wood lice) which become infected with luminous bacteria and live for days as striking luminous organisms.

Luminous copepods and ostracods have been known for many years

and are frequently responsible for displays of sea phosphorescence. The records of mysid luminescence are numerous, and the animals are undoubtedly self luminous, but the group needs further study. These small forms resemble shrimp but can be distinguished by the fact that the carapace is short and fails to cover the entire thorax.

The euphausiads (or schizopods) and the decapods, whose carapace does cover the entire thorax, containing true shrimps and prawns, look alike but differ in the form of the thoracic appendages. They are biramous in the euphausiads (hence the synonym schizopods or split feet), while the decapod appendages are undivided. The luminous species in both these groups are mostly deep sea forms, although they may come to the surface to breed. Their luminous organs are remarkably varied, and the same types are to be found as occur among squid. Thus, some shrimp pour out an abundant luminous secretion from a large gland, as does the squid, *Heteroteuthis*; others possess on various parts of the body true photophores of highly complicated and varied design, containing lenses, reflectors, opaque and color screens, like those of the squid *Abraliopsis*. Photophores are often present on the outer margins of the eye stalks, like the row of photophores on the eyeball of many squid. Finally some species of shrimp frequently become infected with luminous bacteria, although the infection is general and not confined to a gland as in many of the squid observed at Naples and in Japan.

The classification of crustacean groups by C. Zimmer (with luminous orders in italics) is as follows:

Crustacea

Entomostraca

Branchiopoda or Phyllopoda (water fleas) (19 families)

Ostracoda (8 families)

Copepoda (86 families)

Branchiura (*Argulidae*) (3 genera)

Cirripedia (16 families)

Malacostraca

Leptostraca or Phyllocarida (*Nebaliidae*) (4 genera)

Anaspidacea (5 families)

Mysidacea (5 families)

Cumacea (7 families)

Tanaidacea (*Apseudidae* and *Tanaidae*)

Isopoda (24 families)

Amphipoda (61 families)

Euphausiacea or *Schizopoda* (*Euphausiidae*) (11 genera)

Decapoda (76 families)

Stomatopoda (*Squillidae*)

OSTRACODA

General

Ostracods are small crustacea with two hinged valves covering the body. They are found in both fresh and salt water, but only the marine forms are luminous. In a sample of plankton collected in tow nets at night, the ostracods are easily distinguished from dinoflagellates by the puff of bluish luminescent liquid left behind as they swim about in the sea water. Like the copepods they are often responsible for phosphorescence of the sea.

Luminous ostracods appear to have been first recognized by Godeheu de Riville who saw them in 1754 off the Malabar coast and the Maldives. In his "Mémoire sur la mer lumineuse" published in the Mathematical and Physical Memoirs of the Royal Academy of Science in Paris in 1760,¹ he described a sea full of bluish points of light, which, when examined on his finger, turned out to come from small organisms. His excellent figure shows that the animal was undoubtedly an ostracod, probably of the genus *Pyrocypris*, judging from the shape of its shell.

Ostracods were also noted by Tilesius (1819), Baird (1848), Dana (1852), Chierchia (1885), Shepherd (1890), Hansen (1903), Doflein (1906), Kiernik (1908), Lund (1911), and others, but are not listed by Viviani (1805), McCartney (1810), Heinrich (1810), or Quatrefages (1850). Müller (1891) gave the earliest clear account of the light organ and referred to experiments of Chierchia (1885) who was greatly impressed with the brilliance of the light from so small an organism and by the fact that the luminescence lasted for fifteen minutes in alcohol, although it disappeared rather quickly in corrosive sublimate after a preliminary burst of light.

A classification of the Ostracoda by E. Wagler follows. Luminous families and genera are printed in italics.

Ostracoda

*Myodocopa**Cypridinidae* (marine)

Cypridininae (*Cypridina*, *Pyrocypris*, *Crossophorus*, *Codonocera*, *Gigantocypris*)

Philomedinae (*Philomedes*, *Pseudophilomedes*, *Rutiderma*)

Sarsiellinae (*Sarsiella*)

Asteropinae (*Asterope*, *Cyclosterope* and 9 other genera)

Halocypridae (marine)

¹ A translation of Riville's paper appeared in the *Gentleman's Magazine* for 1768, p. 408.

Thaumatoocyprinae (Thaumatoocypris)

Conchoeciinae (Archiconchoecia, ?*Halocypris*, *Conchoecia*, *Euconchoecia*)

Polycopidae (Polycopa, Polycopsis) marine

Podocopa

Cypridae (5 subfamilies, 64 genera) marine or fresh water

Darwinulidae (Darwinula) fresh water

Nesideidae (Nesidea, Bythocypris, Anchistrocheles) marine

Cytheridae (33 genera) marine

Cytherellidae (Cytherella) marine

Present knowledge is not sufficient to warrant the statement that all genera of the subfamily Cypridinae contain luminous species. Of greatest interest is the deep sea form, *Gigantocypris*, an inch in length, which has been obtained on various expeditions. It was caught by the *Challenger* at 1300 to 1600 meters and by the *Albatross*, the *Valdivia*, and the *Discovery*. Chun (1903) wrote that the shell was orange in color with pearl-colored reflectors on the head, but he did not see it luminesce and could not say whether the reflectors were light organs.

The study of Luders (1909) was anatomical and histological, on preserved material, but Luders described a large gland with a reservoir on the upper lip and pores through which a secretion could pass out. There were similarities to the Cypridina gland, in which Doflein (1906) had erroneously described a reservoir. Luders concluded that the *Gigantocypris* gland was probably for luminous secretion but that a coming deep sea expedition must check the possibility by actual observation. Cannon (1931) has filled out the details of internal anatomy but omitted description of the lip organs.

Ostracods are best known for the many biochemical studies of Cypridina, which produces a copious luminous secretion, is easily caught, and can be preserved in the dry state for indefinite periods of time. The genus *Cypridina* was established by Milne-Edwards in 1838 and, as at present constituted, contains 20 to 25 species. The best known is *Cypridina hilgendorffii* Müller, named after a Dr. Hilgendorff, who sent them from Japan to the Berlin Museum. The animal is shown in Fig 95.

Methods of collecting have been published by Kajiyama (1912, 13), and a number of workers during World War II (Haneda, Kuriza, and Nakamura) when Japanese army officers were using the dried *Cypridina* as a source of low intensity light. In close contact with the enemy, where use of a flashlight for night reading of messages would be prohibitive, the officer could place a small quantity of *Cypridina* powder in the palm of his hand, moisten it and read the message by *Cypridina* light.

The animals live in sand at the sea bottom near shore and come

out to feed at night. If a large fish head is tied to a string and hung in the water at night the ostracods collect on it and may be easily picked off when hauled to the surface. Small jars baited with fish flesh and covered with netting, to keep larger fish out, may also be used. For mass wartime collecting, the Japanese set out large earthenware pots, baited with whole fish, resting on a sand bottom at 3 to 10 meters depth. They were tended every two hours between sunset and 1 A.M. Some-

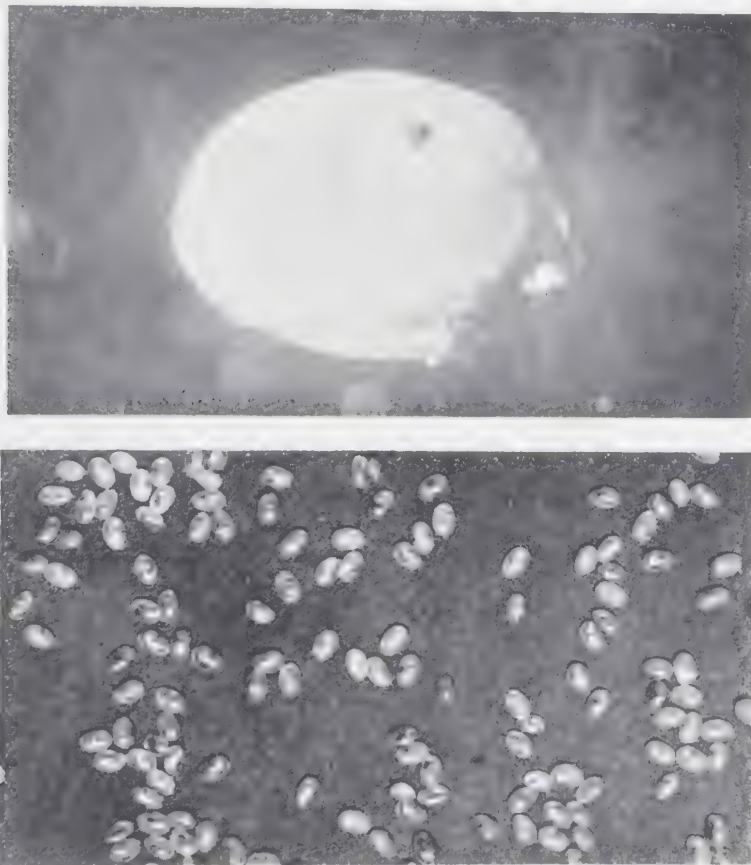


FIG. 95. A single specimen of *Cypridina* (above) and dried animals photographed on a black surface. Original photo.

times a liter of *Cypridina* per pot-trap per hour would be obtained. When the remains of the bait had been removed, the animals were strained from the sea water and placed on blotters in the sunlight to dry or in a heater with the temperature not over 50° C. They must be thoroughly dried and kept dry permanently with large quantities of CaCl_2 to insure brilliant material.

The catches were less on moonlight nights. The animals never feed in the daytime but certain species, at least, react in a definite

manner to light. Haneda (1940) reported observing in the South Sea a small species, *C. noctiluca*, which always responded by secreting luminous material whenever a flashlight was played on the water. He was not certain whether the stimulus of the light flash itself or the mutual impact of Cypridinae responding to the light flash served as the stimulus for secretion of the luminous material, but no other plankton organism responded in this way.

Life History

The life history of *Cypridina hilgendorffii* has been studied by Okada and Kato (1949). The breeding season is March to November at Tateyama. Copulation occurs, and the 40 to 60 eggs develop in the brood pouch of the female. The compound eyes become visible about five days after fertilization in summer and the unpaired median nauplius eye two days later, at which time "a yellow rudiment of the luminous gland of ovoid shape is observed at the upper lip. If the embryo at that stage is pressed down under the cover slip bright luminescence is observed under the microscope." Hatching occurs in ten to twelve days, and after about five moults the adult form is attained. From a length of 0.8 to 0.9 mm on hatching, the adult female becomes 3.7 to 3.9 mm long and the adult male 3.0 to 3.2 mm long.

Histology

The structure and histology of the large luminous gland on the upper lip has been studied by Müller (1891), Watanabe (1897), Doflein (1906), Dahlgren (1916), Yatsu (1917), Okada (1927), and Tagaki (1936). With this array of investigators it would seem that structure should be well known and agreed on. Actually there is disagreement on the number of secretions which are formed. Dahlgren (1916),² Okada, and Tagaki have described four different types of gland cells in *Cypridina hilgendorffii* while Watanabe and Yatsu recognized only two in this same species. Okada found only two types in a species of *Pyrocypis*, and Müller's early description also recognized two types of cells. Doflein mistakenly described a large reservoir for the secretion which was merely a space left behind when the unicellular gland contents had been expelled. Drawings of the gland, according to different workers, are reproduced in Figs. 96, 97, and 98.

Although Müller (1891) studied preserved specimens and made no

² The author brought back fixed *Cypridina hilgendorffii* material from Japan in 1916 which was immediately sectioned and studied by Dahlgren. The results were incorporated only in the privately distributed reprints of his ostracod article, which had previously been published in the *Journal of the Franklin Institute* for 1916.

sections of the animal, his figure of the gland is good and his account of the mechanism of light production of *Pyrocypriis chierchiaie* is correct. He described three groups of gland cells on the upper lip, two groups on the sides, and one in the middle. These produce two kinds of secretion (judging from differences in staining) which are stored in the gland cells and squirted into the sea water through separate openings. There they mix and produce the light. Müller remarked that this view should be confirmed by a study of living

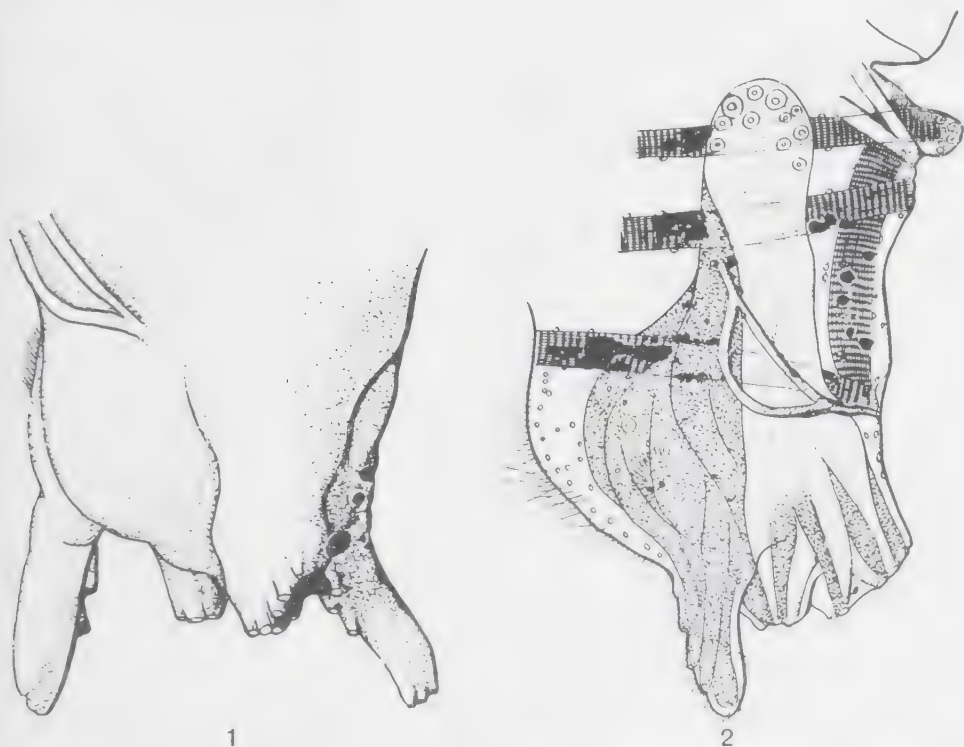


FIG. 96. The upper lip (1) and a (2) section of *Cypridina* luminous gland, after Yatsu. Two types of gland cells are shown.

material, but no clearer description of the actual facts could be given.

Exactly this process has been observed microscopically by Watanabe (1897) and the author (1916) in living *Cypridina hilgendorffii* from Japan. One type of gland is filled with small ($2-3 \mu$ diameter) colorless granules, and a second type with larger (10μ diameter) yellow granules. Both types of granules can be clearly seen as they stream through the pores or gland cell openings and dissolve in the sea water. The drawings of Yatsu (1917) show the intimate relationship of the two types of cells.

Okada (1927) and Tagaki (1936) have both given a minute de-

scription of the organ and the four types of gland cells. The cells open in five groups or papillae, one unpaired anterior and medial and two paired groups, one pair middle and one pair posterior. The four cell types are:

1. Cells with large granules, yellow in color, 5–10 μ in diameter dissolving in Bouin fluid, not staining in hematoxylin, eosin nor in Heidenhain's iron hematoxylin.



FIG. 97. Section of *Cypridina* luminous gland, after Okada. Four types of gland cells (gl) are shown, as well as the anterior and median protuberance (Pr At, Pr Ct); the mouth, O, and supraesophageal ganglion, Ge.

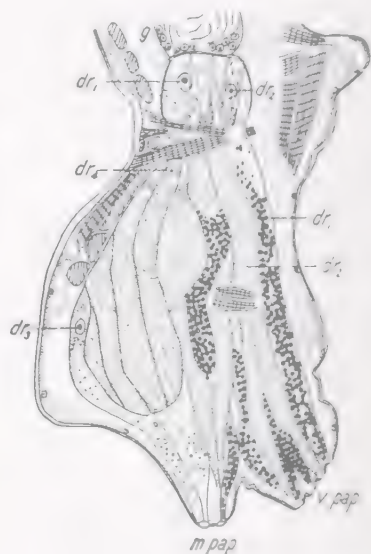


FIG. 98. Section of *Cypridina* luminous gland, after Tagaki. Four types of gland cells (dr) are shown as well as the median and anterior papillae (m pap, v pap) and supraesophageal ganglion, g.

They are found in two symmetrical masses of about five cells each, reaching back to the supraesophageal ganglion. They empty by all five papillae.

2. Cells with fine granules, staining in eosin and hematoxylin, arranged in two groups, one of eighteen and one of two cells, not reaching the supraesophageal ganglion. They open on the anterior and the paired middle papillae.

3. Cells, four to five in number and brownish in color with medium-sized granules, on the oral side of the organ, opening by the middle pair of papillae. Each granule is surrounded by a clear zone. Although Dahlgren described them as fat-like, Okada found that they did not stain in Sudan III.

4. Very numerous mucous cells in two groups of sixteen to eighteen each, on

the oral side of the upper lip and opening at the end of the posterior pair of papillae. These cells contain mucous granules and a fine protoplasmic network.

The description of the gland morphology by Yatsu (1917) agrees with that of the above authors except that he found only "mucous" cells opening into all five papillae and "yellow" cells opening into the middle and anterior papillae. The mucous cells stained deeply in methyl green and resembled gland cells of the appendages and the shell. The yellow cells were sometimes 0.7 mm long and their granules 10 to 15 μ in diameter and frequently angular.

Luciferin and Luciferase

For chemical investigation ostracods are by far the most favorable animals which exist. The large luminous glands, the abundant formation of secretion, and the fact that, when quickly dried, the ability to luminesce on moistening is retained indefinitely² make these organisms unique. The author's attention was first directed to this animal in 1916, during a visit to the Marine Laboratory of the Tokyo Imperial University, at Misaki, Japan. At that time the presence of luciferin and luciferase in ostracods was demonstrated by the method of Dubois (1885), and the general chemical behavior was studied. At one time, the author (1916, 17) suggested the name of photophelein (from the Greek *photos*, light, and *phelein*, to assist) for luciferin and photogenin (from the Greek, *photos*, and *genao*, to produce) for luciferase but soon (1918) abandoned these terms. No appreciable heat production and no CO₂ can be detected when luciferin and luciferase are mixed (Harvey, 1919), but the concentration of the two is undoubtedly very low.

One hundred cubic centimeters of a sample of dried Cypridinae weighs about 24 grams and contains about 20,000 individuals, each 2 to 3 mm long. Each dried animal therefore weighs a little over a milligram. The great volume of dried material is due to the chitinous shells or valves which remain stiff while the body enclosed within the valves shrinks to a very small mass. Despite the small size, the luminous material is capable of giving a light, easily visible when distributed through a large quantity of sea water. The author (1923) determined that crude extracts containing only one part of dry Cypridina in 400,000,000 parts of water will luminesce sufficiently to be detected by the dark-adapted eye. If the dried animal contains 1% luciferin, the concentration would be 1:40,000,000,000. Such a dilution is comparable to the concentration (1:10,000,000,000) in

²Some of the early dried material is still on hand, potentially luminous after thirty years.

which the fluorescence of compounds like fluoresceine can be detected or the chemiluminescence of compounds like Luminol (amino phthalichydrazide). The dark-adapted eye is so sensitive to small amounts of light that a solution containing a trace of luciferin may appear brilliant. Cypridina itself selected an excellent method of light production, but the small amounts of material involved have made chemical procedures of purification difficult.

When the dried Cypridinae are ground in a mortar and placed in water, the powder exhibits bluish luminescent specks of gland material and trails of luminescence as the photogenic substances go into solution. It is not feasible to separate dried gland from other parts of the body, but the dry powder can be extracted with water and other solvents, and crude extracts of luciferin and luciferase can be prepared. It must not be forgotten that these crude extracts contain all the substances of a whole animal which can dissolve in water or in the solvent used for extraction. Therefore much of the early work of Harvey (1917, 19) and Kanda (1920, 21, 24) on precipitation of luciferin and luciferase by various reagents or by salting out methods must be repeated with purer extracts since both luciferin and luciferase may come out of solution adsorbed on proteins and other precipitates. Many of the chemical tests applied to crude solutions to determine the nature of luciferin and luciferase are of little value, and later work with partially purified material has indicated that luciferin and luciferase in crude Cypridina extracts behave very differently from the purified material.

Relation to Oxygen

One of the difficulties in isolation of luciferin is the ease with which the substance oxidizes. As with most luminous animals oxygen is necessary for luminescence, but in such small amounts (Harvey, 1917, 20) that Kanda (1920) once doubted that the reaction was an oxidation. Although the oxygen tension-luminescence intensity curve has not been determined, it is known that light will appear in lower oxygen tensions than in the case of luminous bacteria. The author (1927), using crude extracts, made an attempt to determine the molecules of oxygen necessary to produce one quantum of light at a wave length of 0.48μ , the maximum of the Cypridina spectrum, and arrived at the figure 88, but this cannot be considered too accurate.

Not only does Cypridina luciferin oxidize in presence of luciferase with light production, but it also oxidizes in absence of luciferase, without light production. In purification procedures this spontaneous oxidation of luciferin must be prevented, either by working in complete

absence of oxygen or by converting the luciferin to a compound that will not oxidize.

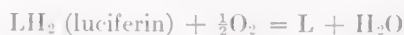
Oxidized Luciferin and Reversibility of the Reaction

The author's (1918, 20) attention was first directed to the possibility of reducing the oxidation product of luciferin, which was named "oxyluciferin," by the observation that a test tube of clear solution of crude Cypridina luciferase (which must contain the products of oxidation of luciferin), although it may give off no light at first when shaken with air, after standing a day or so emits quite a bright light if disturbed. This was especially true when the luciferase had become turbid and ill-smelling from the growth of bacteria. Thinking that the bacteria produced a substance which could be oxidized by the luciferase, the author tried growing bacteria and also yeast on appropriate culture media, and, after some days of growth, mixing the culture media containing the products of bacterial or yeast growth with luciferase, expecting to obtain light; but no light appeared. However, if a little crude luciferase solution was added to the bacterial or yeast cultures and they were then allowed to stand a short time, light appeared whenever they were shaken. Indeed, such cultures behaved much as a suspension of luminous bacteria which has used up all the oxygen in the culture fluid and will only luminesce when, by agitation, more oxygen is dissolved. The effect turned out to be due to the anaerobic conditions brought about by the bacteria, as a result of which the "oxyluciferin" in the luciferase solution was reduced to luciferin. The reducing action of bacteria and yeast is well known. Hayashi and Okuyama (1929) and Nakamura (1940, 47) have also observed such reducing reactions.

Many methods of reduction which involve the addition of hydrogen can also be used—finely divided platinum or palladium in presence of hydrogen and active hydrogen evolved from dissolving metals, such as magnesium powder or zinc dust. If a freshly cut surface of aluminum, magnesium, manganese, zinc, or cadmium is placed in a solution containing oxyluciferin and luciferase, the surface will luminesce (Harvey, 1923). This is the result of reduction of "oxyluciferin" by a layer of nascent hydrogen near the surface of the metal and reoxidation with light production in a more external layer. For the same reason, light appears at the cathode when a solution of "oxyluciferin" and luciferase is electrolyzed. Sodium hydrosulfite ($\text{Na}_2\text{S}_2\text{O}_4$), hydrogen sulfide, ammonium sulfide and other reducing agents are effective in reforming luciferin from its oxidation product. The author (1926) was particularly impressed with the activity of sodium hydrosulfite as a reducing agent for "oxyluciferin" and observed that mild oxidizing

agents like ferricyanide very rapidly reoxidize the luciferin, but without light production. Acidity and light favor the reduction according to experiments of the author (1920) and Araki (1950).

Since all methods of reducing "oxyluciferin" are similar to those by which dyes like methylene blue can be reduced, the oxidation of luciferin was compared to the oxidation of a leuco-dye, particularly methylene white to methylene blue. The oxidation of this dye takes place spontaneously in presence of molecular oxygen and more rapidly in alkaline solution. Chase (1940) has found that rates are almost identical, that both luciferin and methylene white oxidation are roughly proportional to the fifth root of the OH-ion concentration. Since neither the "oxyluciferin" nor the oxidized methylene blue can be reduced by mere removal of oxygen in solution with an air pump the change on oxidation cannot be similar to that of hemoglobin and oxyhemoglobin. It is not an oxygenation but may be expressed as:



It was also observed that the reduction of "oxyluciferin" was not complete and would not occur at all if the "oxyluciferin" solution stood in contact with air for too long a time. In this respect it differed from the methylene-blue system, and attempts (1927) to determine a redox potential in crude solutions gave no consistent results. The luciferin-oxyluciferin system did not appear to be a completely reversible one.

The situation has been cleared up by Anderson's (1936) work, using purified luciferin on which quantitative determinations of reduction were made. Two important facts regarding the oxidation were established. First, that the oxidation with light production in presence of luciferase gives an oxidation product which cannot be reduced. It is the oxidation without light production, taking place spontaneously or with oxidants like potassium ferricyanide, which is reversible. Second, that the change with ferricyanide occurs in two steps, one the reversible oxidation previously referred to, the second irreversible, and probably also an oxidation, although this has not been definitely demonstrated. The spontaneous oxidation of luciferin without emission of light in crude solutions (without luciferase) is probably catalyzed by traces of heavy metals in the solution and proceeds much more slowly when the luciferin has been purified. Both the dark oxidation and the luminescent oxidation must take place simultaneously when luciferin is mixed with luciferase.

The redox potential of the first step has been placed by Anderson (1936) near the hydroquinone-quinone system but 0.01 - 0.005 volt more negative. Korr (1936) also considered it near quinone. If a

two-electron change is assumed, the redox potential would be $E_0' = +0.26$ volt at pH 7.0, about halfway between quinone and orthochlorophenol-indophenol.

The reversible product has been designated by Anderson as oxidized luciferin, a name indicating that it is the oxidant (while luciferin is the reductant), since "oxyluciferin" implies that the change is an oxygenation. The nature of the irreversible oxidation product formed during the luminescent reaction with luciferase is unknown. Van der Kerk (1942) has called the reversibly oxidized luciferin (in bacteria) dehydroluciferin.

Specificity of Luciferin and Luciferase

After the discovery of luciferin and luciferase in *Cypridina* the author (1922, 26, 28) made many experiments directed to finding a means of oxidizing *Cypridina* luciferin with light production in absence of *Cypridina* luciferase. The materials tested included extracts of many luminous and also non-luminous animals, extracts which must have contained the iron respiration catalyst or the yellow respiration enzyme; plant extracts (potato and turnip juice) containing oxidases peroxidases and catalases, tried with and without H_2O_2 , and laked blood containing hemoglobin, with and without H_2O_2 . Not one of these extracts, nor luminous tissue from twenty-four different luminous groups, produced light when mixed with *Cypridina* luciferin. The only foreign extracts capable of oxidizing *Cypridina hilgendorffii* luciferin was luciferase from another genus of ostracods, *Pyrocypris* luciferase, and another species of *Cypridina*, *C. Norvegica*. Negative results with luciferin were also obtained with a long list of oxidizing agents from all positions on the redox scale, permanganates, bichromates, ferricyanides, barium peroxide, benzoyl peroxide, perchlorates, persulfates, perborates, hypochlorites, hypobromites, hypiodites, and various heavy metal catalysts such as iron, copper, and manganese. Although oxidation of luciferin may occur, no light is emitted.

A similar attempt to find easily oxidizable substances which will luminesce with *Cypridina* luciferase also failed. Included were heated extracts of many luminous forms which might contain luciferin, compounds known to exhibit chemiluminescence, such as various oils, esculin, lophin, amarin, aminophthalichydrazid (Luminol), dimethyldiacridinium nitrate, pyrogallol and many easily oxidizable amino- and hydroxyphenol compounds; also many fluorescent dyes. The same specificity that prevents luciferin of one form from luminescing with the luciferase of another probably prevents these easily oxidizable compounds from giving light.

The luciferin of another genus of ostracods, *Pyrocypis* will luminesce with *Cypridina* luciferase, as does *Pyrocypis* luciferase with *Cypridina* luciferin. Experiments (1924) in which the color of the light appeared to be determined by the luciferase may be open to another interpretation.

Purification of Luciferin

Methods of purification have been published by Kanda (1924, 29) and by Anderson (1935). Kanda's method is briefly as follows. After preliminary treatment of dry *Cypridina* with (1) petroleum ether, (2) ether, and (3) benzene to remove fat, the fat free material is extracted with absolute methanol which removes the luciferin. This solution, after filtering off the *Cypridina* residue is evaporated to a syrup and a large excess of absolute ethanol added when another precipitate forms which is filtered off. The luciferin is in the ethanol, which is evaporated to a syrup, and benzene is added. Another non-active precipitate forms, and the benzene filtrate is found to contain the luciferin, which has in the course of the extraction procedure become soluble not only in benzene but also in petroleum ether, ether, and chloroform. Kanda believed that the luciferin in the animal was in some way associated with a protein, which was soluble in the alcohols and became dissociated from the protein during the extraction treatment when it dissolved in the benzene. However, Anderson (1935) found solubility in benzene only under limited conditions, and Chase (1948) has reported that purified luciferin is insoluble in benzene, ether, and petroleum ether.

Kanda (1930) found nitrogen and phosphorus in his purified material and was led to the idea that it was phospholipid. He (1932) later described crystalline luciferin. It was obtained from a concentrated ethyl alcohol solution of luciferin from which extraneous material had been removed by precipitation with CdCl_2 and the residue dissolved in ether or benzene and washed with water. Kanda wrote: "From this ethereal or benzene solution in a large beaker, slowly evaporated and dried in air, *Cypridina* luciferin crystallizes in rosettes which are insoluble in acetone. It also crystallizes from alcohol but with difficulty. The crystalline luciferin still contains phosphorus." This crystallization has never been confirmed.

The Anderson (1935) method involves a complicated series of chemical manipulations with two cycles of benzoylation and hydrolysis and results in a product on which reliable absorption spectrum measurements can be made. This purified luciferin made up 0.24% by weight of the *Cypridinae*. After only one cycle of purification 1 mg

of the material emitted 13 to 30 light units and 1 mg of the doubly purified luciferin emitted 40 to 60 light units, while 1 mg of the original dried cypridinae emitted only 0.021 to 0.033 unit, all tested under standard conditions. Hence in terms of activity, i.e., light emission, the purification was some 2000-fold.

In order to follow the various steps in purification a method of assay is necessary. Kinetic studies on *Cypridina* luminescence have indicated that under standard conditions the total light emitted is a measure of the luciferin present. This total light can be readily

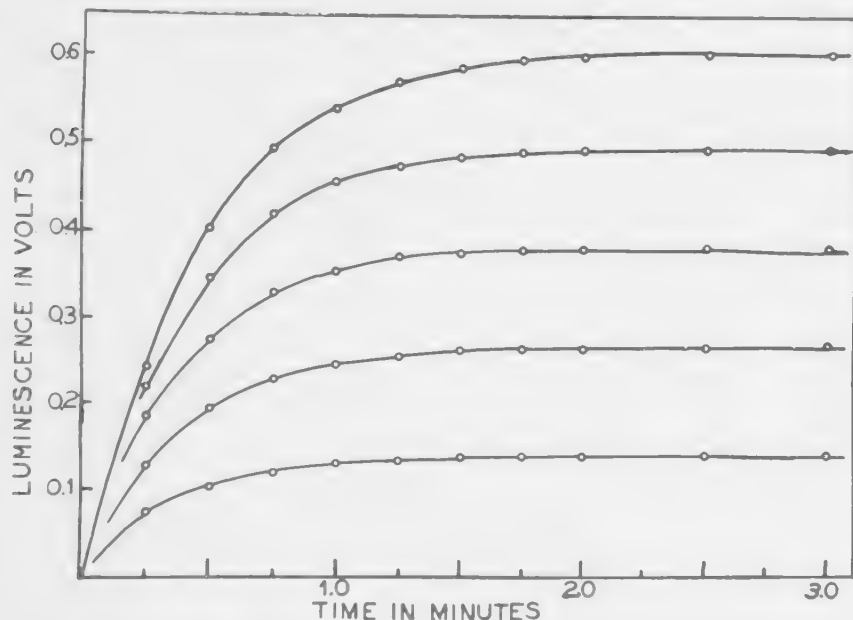


FIG. 99. Total light emitted (in arbitrary units) vs. time for different concentrations of luciferin. After Chase.

measured by a device perfected by Anderson (1933), in which a photoelectric cell detects the light and stores the electric energy in a condenser. The charge on the condenser is then measured by a potentiometer and Lindemann electrometer as a voltage which tells the total light emitted at any instant. If the reaction is relatively slow, curves shown in Fig. 99, may be plotted to show the course of the reaction.

Anderson's method of purification has been summarized by Chase (1948) as follows: "Dry, powdered organisms are extracted for twenty four hours with absolute methyl alcohol that is kept free of dissolved oxygen by saturation with purified hydrogen in a special extraction vessel. The vessel is then opened and a small amount of *n*-butyl alcohol is added and the methyl alcohol is removed by evapora-

tion at low pressure. Butyl alcohol containing some solid material remains. The butyl alcohol is now decanted and the remaining solid is washed several times with butyl alcohol and the washing added to the butyl alcohol solution originally decanted. This solution, after having been chilled, is treated with benzoyl chloride. The resulting derivative of luciferin not only does not give light on addition of luciferase, but is much more stable in the presence of air than is the luciferin in its original state. Water is now added to hydrolyze the excess benzoyl chloride, and the butyl alcohol fraction is dissolved in 10 volumes of water. The resulting solution is extracted with ether, into which most of the butyl alcohol and luciferin derivative pass, leaving highly colored impurities in the aqueous phase. The ether is next removed *in vacuo*, leaving the inactive luciferin, already considerably free of colored impurities, in the residual butyl alcohol. This solution is mixed with a large volume of 0.5 *n* HCl, saturated with hydrogen, and heated in a hydrogen atmosphere for an hour at 95–100° C and then cooled in an ice water bath. The mixture, now containing active luciferin, is then washed again with ether. At this stage, the "reactivated" luciferin remains almost entirely in the aqueous phase, and considerable colored material passes into the ether phase. The luciferin is finally extracted from the aqueous solution with *n*-butyl alcohol, deaerated with hydrogen, and, if desired, is put through the same cycle of purification a second time."

In all steps where oxygen is present the solutions are kept cold by ice water baths. Some yellow color remains, a property of luciferin itself. This method results not only in a purification of the luciferin some 2,000 times, but the stability of the luciferin against oxidation is also greatly increased, perhaps because oxidizing agents that were present in the original material have been removed during the purification procedure.

Properties of Purified Luciferin

The chemistry of Cypridina luciferin has recently been reviewed by Chase (1948). Only a brief outline of properties will be given, and the early work of the author (1917, 19) and of Kanda (1920, 21, 24), based on crude extracts, will be omitted.

The purified luciferin obtained by Anderson's method is decreasingly soluble in the series of solvents—water, methyl, propyl, butyl, ethyl, and amyl alcohols, aniline, acetone, and chloroform, and insoluble in ethyl ether, petroleum ether, and benzene. It will dialyze through parchment or collodion and is adsorbed on certain surfaces so that chromatographic methods can be applied for purification.

Phosphorus has been found in the purified luciferin, and some of it apparently becomes inorganic phosphorus on oxidation (McElroy and Ballantine, 1944). Chakravarty and Ballentine (1941) found no sulfur, nitrogen, ash, or halide in the purified luciferin, but more recent work (Chase and Gregg, 1949) indicates that nitrogen is probably present. Presence of phosphorus has not been confirmed by Mason (1951).

The purified luciferin will combine irreversibly with cyanide (Giese and Chase, 1940), and in this condition no luminescence appears in presence of luciferase. If the cyanide effect represents cyanhydrin formation, an aldehyde or keto group is indicated in the luciferin molecule. When sodium azide is added to luciferin, a reversible inhibition of the luminescence of luciferin and luciferase occurs (Chase, 1942) which has been attributed to a molecule of a hydrazoic acid combining with one molecule of luciferin, possibly indicating azide hydroquinone formation (Chase, 1948).

Effect of Light

Cypridina is fundamentally nocturnal in habit, remaining in sand during the day, but is quite capable of ejecting a luminous secretion in bright sunlight. There is no such inhibition by daylight as occurs in ctenophores. Nevertheless, the author (1925, 26) demonstrated that the luminescent reaction is light sensitive. If the crude luminescent solution of luciferin and luciferase is placed in a small test tube and partly exposed to the intense beam of a carbon arc, filtered through water to prevent heating effects, the light will disappear in the exposed region in a matter of seconds. Oxygen is necessary, and the blue and near ultraviolet rays are the effective ones, but red, yellow, and green wave lengths are also effective in presence of oxygen if photosensitive dyes like eosin, erythrosin, rose bengale, cyanosin, acridine, or methylene blue are added. Luciferase is not changed by the light exposure, but the luciferin is rapidly oxidized and disappears without luminescence emission.

Photosensitization is responsible for the effect of blue light also. Chase (1940), using purified samples of luciferin, found no effect of blue light unless riboflavin was added. The apparently spontaneous oxidation of the luciferin on illumination in crude solution is due to some impurity, probably riboflavin, which acts as photosensitizer. Chase and Giese (1940) have discovered that luciferin is destroyed by ultraviolet light of short wave lengths, 230 to 280 $m\mu$, and that luciferase is also destroyed by these wave lengths. Both substances have

absorption bands in this region, and in both cases the destruction takes place in absence of oxygen.

Relation to Adenosine Triphosphate

At the time McElroy and Ballentine (1944) demonstrated the presence of labile phosphorus in highly purified¹ luciferin, they suggested that a phosphorylation was a necessary step in supplying the luciferin-luciferase reaction with sufficient energy to radiate in the blue spectral region. Later studies by McElroy (1947) and McElroy and Strehler (1949) have demonstrated the importance of energy-rich phosphate compounds in fire fly luminescence. The addition of adenosine triphosphate (ATP) to an extract of fire-fly lanterns whose light has disappeared will revive the luminescence in a striking manner. However, a similar experiment carried out with dried *Cypridina* extracts (crude luciferase) gives negative results. All attempts to link ATP with *Cypridina* luminescence have failed. It was thought at first that the dried material had lost some necessary transphosphorylase, but negative results have also been obtained with fresh *Cypridina* extracts, an experiment carried out at the author's request by Dr. Yata Haneda in Japan.

Structure of Luciferin

Cypridina luciferin has been regarded at various times as a protease (Harvey, 1919), a phospholipin (Kanda, 1930), a polyhydroxybenzene (Anderson, 1936; Korr, 1936), a reduced quinone, naphthoquinone or anthroquinone (Chakravarty and Ballantine, 1941; Van der Kerk, 1942) and a flavin (Johnson and Eyring, 1944). That the molecular weight is low seems certain, although calculations of its exact value depend on the purity (unknown) of the luciferin sample. Giese and Chase (1940), from studies on cyanide and azide (1942), and particularly on oxidation with ferricyanide (Chase, 1949), have reached a value of 250 to 500 as the most probable for molecular weight.

The most highly purified luciferin preparations are yellow in color. Spectral absorption studies of Chase (1940, 43, 45) show that after a preliminary shift in maximum absorption from 435 $m\mu$ to 465 $m\mu$, the 465 $m\mu$ absorption band becomes less and less apparent and disappears entirely in the course of a few hours. Oxidized luciferin is colorless. In presence of luciferase, the same changes take place but they are about 100 times as rapid, the increased velocity depending on the concentration of luciferase. Luminescence is still emitted by the solution

¹ It is not yet certain whether phosphorus was present in some impurity or in the luciferin.

after the 435-m μ band has disappeared. The curves are shown in Fig. 100.

In the ultraviolet, Chase (1950) has observed in a phosphate buffer pH 6.8 or a methanol solution of the highly purified luciferin, a sharp maximum at about 265 m μ , a shoulder at 310 m μ , and broad absorption extending into the visible to the maximum at 435 m μ . On standing in phosphate buffer the 265 m μ peak decreases markedly

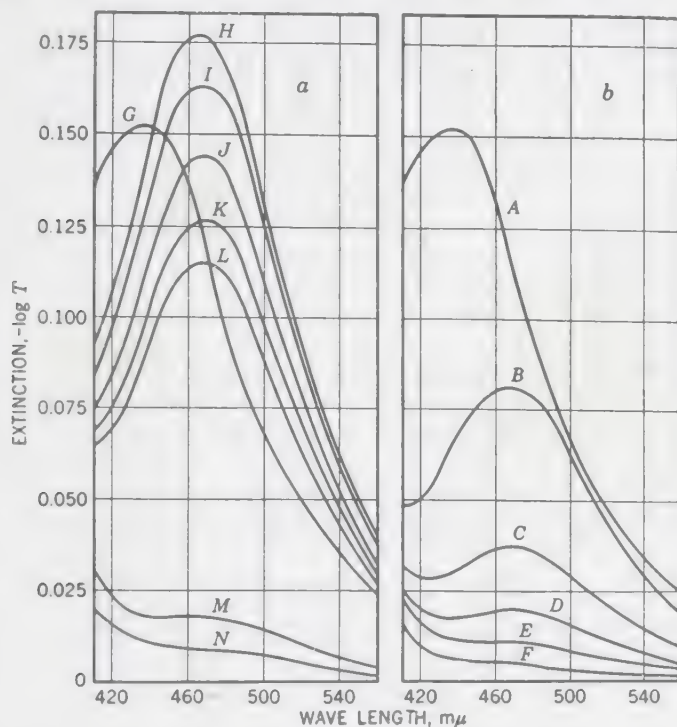


FIG. 100. Spectral absorption curves of purified luciferin, after Chase in the *Journal of Biological Chemistry*. At left the change during spontaneous oxidation and at right the oxidation in presence of luciferase is shown. G and A are curves before oxidation has occurred and the letters that follow are curves for succeeding time intervals. From *Advances in Enzymology* by courtesy of Interscience Publishing Co. Inc.

and a new absorption band appears at 365 m μ and subsequently disappears. These changes are quantitatively related to the ability of luciferin to luminesce with luciferase. Luminescence no longer appears after the 265 m μ peak decreases to a stable density value.

The data at hand show no difference in absorption change of the spontaneous oxidation and the luciferase catalyzed oxidation. The position of the visible absorption bands is in agreement with a quinonoid or anthraquinonoid structure. The initial shift from an absorption maximum at 435 m μ to 465 m μ might be correlated with a reversible

redox change similar to that in the system, hydroquinone quinone while the subsequent disappearance of the 465 $m\mu$ band might represent the irreversible change. The question remains open as to whether the irreversible oxidation is merely a second step of oxidation comparable to other well known, two-step oxidations, or whether it represents the opening of a ring structure without oxidation, or whether it represents the irreversible oxidation of another group on the luciferin molecule. Johnson, van Schouwenburg, and van der Burg (1939) have suggested the latter as a possibility.

Chakravarty and Ballentine (1941) proposed the structure for luciferin shown in Fig. 101, leaving open the question as to whether

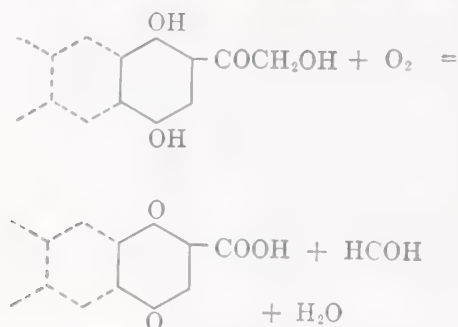


FIG. 101. A possible structure of luciferin, showing a reversible and an irreversible oxidation of the molecule.

benzene, naphthalene, or anthracene rings were involved and the actual point of attachment of the side chain. The two hydroxyls in the 1-4 position were believed to be involved in a change to a quinone structure in the reversible oxidation, while the $\text{CO-CH}_2\text{OH}$ group was believed to go to COOH in the irreversible oxidation.

Van der Kerk (1942) has been even more specific, suggesting a 1-4-naphthohydroquinone with a $\text{CO-CH}_2\text{OH}$ group in the 2 position as the structure of bacterial luciferin, and Spruit (1946, 47) has made a detailed study of the absorption spectra of a large number of substituted naphthoquinones to compare them with the action spectrum of bacterial luminescence (Kluyver, van der Kerk, and van der Burg, 1942). Spruit (1946) and Rexford (1949) both synthesized the hydroxymethylketone compound, and Johnson, Rexford, and Harvey (1949) and Spruit (1949) have tested it on Cypridina luciferase. The synthesized product dissolves in water with a yellow color but will give no luminescence when mixed with luciferase. Like other naphthoquinones, it also has a strong inhibiting effect on the luminescence of luminous bacteria. Luciferin is certainly not 1-4-dihydroxynaphthyl 2-hydroxymethylketone, but might be a related compound.

However, a large number of substituted naphthoquinones have been tested with negative results.

Another suggestion (Johnson and Eyring, 1944), that Cypridina luciferin is a flavin appears to have little in its favor (Anderson and Chase, 1944; McElroy and Ballentine, 1944), although it must not be forgotten that riboflavin is present in high concentration in the lanterns of the fire-fly (Brooks, 1940; Ball and Ramsdell, 1944) and in the luminous material of an earthworm (Backovsky, Komarek, and Wenig 1939; Wenig, 1946). The last authors have stated that the luminescence of the earthworm is the result of chemiluminescence of riboflavin, which changes to lumiflavin.

Properties of Oxidized Luciferin

No specific chemical investigation of oxidized luciferin has been made. It has generally been assumed that its properties differ little from those of luciferin. In view of the final irreversibility of the oxidation, whether spontaneous or luciferase-catalyzed, much needed studies on this compound or compounds should be undertaken.

Luciferin-Luciferase Combination

There is considerable evidence that luciferin can combine with luciferase. The finding of Kanda, already mentioned, that luciferin in dried Cypridinae changes in properties on alcohol treatment is evidence for combination with some material in the animal. It has already been pointed out that highly purified luciferin is very soluble in all the alcohols from methyl to amyl and in acetone and chloroform. If the dried Cypridina material is extracted with the same solvents, the luciferin which it contains is found to be readily soluble in methyl alcohol, but only slightly so in ethyl, propyl, and butyl alcohols and practically insoluble in amyl alcohol, acetone, and chloroform (Chase, 1948). Previous treatment of the dry Cypridina powder with benzene to remove fat, increases slightly the amount of luciferin extractable in all solvents but does not greatly alter the order of solubility. Perhaps it is combination of luciferin with luciferase in the dried animal which makes it insoluble in such solvents as amyl alcohol, acetone, and chloroform.

The inability of luciferin to be reduced after long standing in aqueous solutions in presence of oxygen has already been stressed. It has been observed (Johnson and Eyring, 1944) however, that a luciferase solution, which might also contain oxidized luciferin, after dialysis for many hours against distilled water and preservation at low temperature for many days, will luminesce slightly, if hydrosulfite is

first added and the solution is then shaken with air. The hydro-sulfite may have reduced oxidized luciferin bound with luciferase, which did not dialyze away and did not undergo irreversible oxidation over this long time period.

Finally, conclusive proof of combination of luciferase and luciferin is to be found in recent work of Chase (1949). Using concentrations of luciferin which varied 200 fold and a low concentration of luciferase, Chase obtained a value of about 10^{-6} for the Michaelis constant, i.e., the equilibrium constant for the enzyme-substrate combinations, K_m . This is an order of magnitude similar to that found for other oxidative enzyme systems. The data indicate that one molecule of luciferin combines with one molecule of luciferase. Recently the effect of two temperatures (15° and 22°) on the Michaelis constant has been studied by Kauzmann, Chase, and Brigham (1949), and certain thermodynamic constants relating to the formation and decomposition of the enzyme substrate complex have been calculated. Rise of temperature increases the dissociation of the complex.

Purification of Luciferase

The principal recent purification of this material that has been attempted involves several days dialysis against distilled water of a cold water extract of dried powdered Cypridina at low temperature, until the extract, originally brownish, becomes almost colorless. Considerable inactive protein is precipitated and dialyzable compounds are removed. The luciferase does not pass the dialyzing membrane. This material is not by any means pure, as has been shown by electrophoretic measurements in a Tiselius apparatus (Chase, Schryver, and Stern, 1948). One stationary and two mobile components were found at a pH 7.6. The stationary component had no luciferase activity and contained little or no nitrogen. It might be glycogen. The mobile components migrated as anions at pH 7.6, with electrophoretic mobilities of 10 and 17×10^{-5} cm² sec⁻¹ volt⁻¹. The luciferase activity was probably associated, or migrated, with the component of lower mobility.

Properties of Luciferase

Apart from destruction by heat and ultraviolet light, non dialyzability, and the general properties of protein enzymes, the more specific properties of luciferase are little known. Early experiments of Harvey (1919) and Kanda (1919, 21) using extracts of whole Cypridinas, that indicated practically complete precipitation with phosphotungstic, picric, and tannic acids, basic lead acetate and saturated (NH₄)₂SO₄.

should be repeated with quantitative control. Fractional salting out experiments would be most important. The definite formation of an antiluciferase (not destroyed at 61°C but thermolabile at 71°C), when luciferase is injected into the blood of a rabbit, would indicate protein nature or very close association with a protein (Harvey and Deitrick, 1930). Destruction of luciferase by trypsin is further evidence of protein nature.

Luciferase is readily adsorbed on surfaces. In fact this property has interfered with attempts by Chase (unpublished) to separate luciferase by high-speed centrifuging in steel rotors. Nothing is known of its molecular weight or of the possible presence of heavy metal components.

Kinetics of Cypridina Luminescence

A study of the time course of light emission when *Cypridina* luciferin and luciferase are mixed has afforded a great deal of information on the behavior of luciferase. It is in fact a convenient and rather unique method of studying enzyme kinetics because the light intensity at any moment is proportional to reaction velocity at that moment. The velocity can be determined directly rather than by the usual method of measuring amount of substrate transformed or amount of reaction products which have accumulated. Moreover the instruments for measuring and recording light intensities are highly developed and highly sensitive.

Methods. The first studies of *Cypridina* luminescence kinetics were made by Amberson (1922) and served to establish the fundamental laws involved. The method was to record on moving photographic film the light which passed through a narrow slit from a vessel in which the luciferin and luciferase solution were suddenly mixed. After development of the film, a streak of blackened emulsion was obtained from which, by proper calibration and film density determination, the light emitted during the course of the reaction, i.e., the decay of luminescence, could be determined. The experiments of Amberson were continued by Stevens (1927), using relatively weak luciferase solution to obtain long-lasting luminescences (half decay time of 24 seconds), which could be read by visual photometric methods (Machbeth illuminometer). Curves are shown in Fig. 102. Harvey and Snell (1930) used high concentrations of luciferase to obtain very short flashes of luminescence (half decay time 0.5 to 1 second), which were automatically recorded on moving paper with photocell detection, amplification, and string-galvanometer technique. A record is shown in Fig. 103. Finally, a study (Chance, Harvey, Johnson, and Milli-

kan, 1940) of the rate of development of light (see Fig. 106) has been made, recording luciferin, luciferase, and oxygen when mixed under various conditions, using a special, rapid (0.001 second) mixing device, photocell detection, amplification and photography of the record on a cathode ray oscillograph screen. This technique records the development as well as the decay of light. Unfortunately all the above work on Cypridina has been carried out on crude solutions of luciferin and luciferase. Since these solutions are merely extracts of the whole

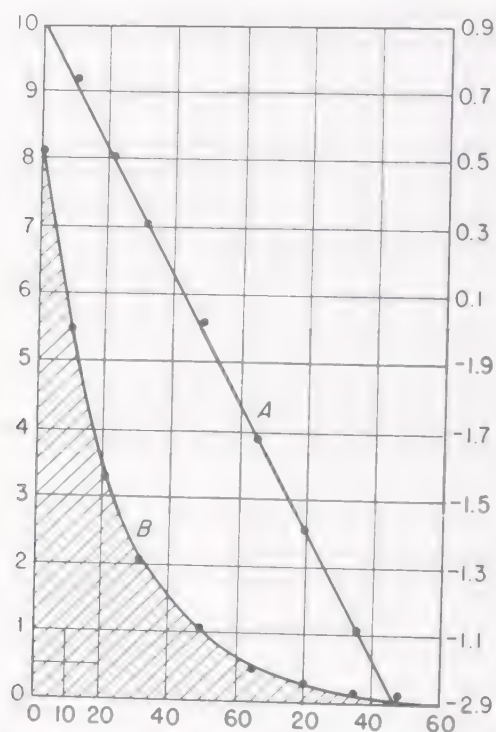


FIG. 102. The decay curve of luminescence, *B*, when Cypridina luciferin is mixed with small amounts of Cypridina luciferase. Luminescence intensity vertical, time in seconds horizontal. The reaction is half complete in 17 seconds. The area of the curve measures the total light emitted. Note that plotting log luminescence intensity against time, *A*, gives a straight line. After Stevens.

dried Cypridinas they contain many other substances than luciferin and luciferase and a considerable and variable amount of brownish pigment. Although general facts were established, certain peculiarities appeared that are not observed with the purified luciferin and luciferase. For example, all the older records indicated that the velocity constant of the reaction varied with luciferin concentration, a situation that should not be true if the reaction is of first order. A repetition of some of these experiments by Chase and Harvey (1942) using purified Cypridina luciferin and luciferase indicate no change

in velocity constant when the initial luciferin concentration is varied.

General Laws. The various studies on kinetics all indicate that light intensity decreases logarithmically with time as shown in Fig. 102. A plot of log light intensity *vs.* time is a straight line with the exception of the first few seconds, where the light intensity is greater than expected, an effect known as the initial flash. The luciferase catalyzed oxidation of luciferin behaves like a unimolecular reaction in which light intensity is a measure of reaction velocity, dx/dt . The velocity constant, k , is independent of luciferin concentration and directly proportional to the luciferase concentration. The velocity constant is in fact a measure of luciferase under standard conditions.

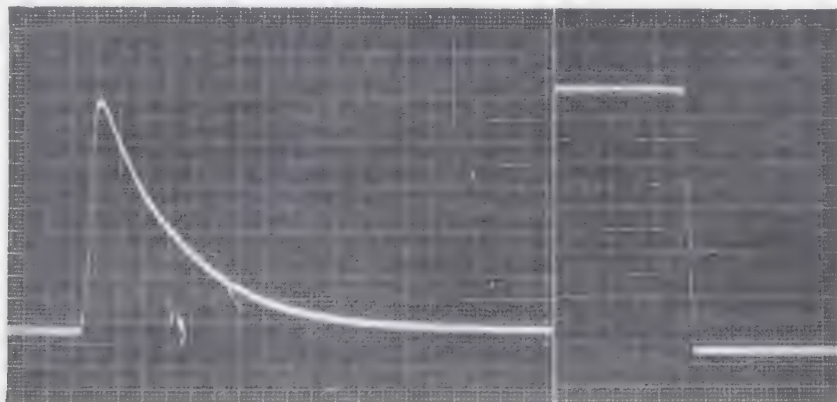


FIG. 103. Left, string galvanometer record of light intensity in arbitrary units (vertical *vs.* time, when luciferin and concentrated luciferase are mixed. Right, delay in response of string when potential is applied. The large time units (horizontal) are 0.2 second. After Harvey and Snell.

The area under such decay curves represents the total light emitted. With the light integrating apparatus used in all investigations of Anderson and of Chase the total light emitted up to any instant of time can be read during the course of the reaction. It is quite certain that under standard conditions total light emitted is a measure of initial luciferin concentration and can be used as a convenient method of assay. With changing luciferase concentrations the total light is the same, but the rate at which the light is emitted varies with the luciferase concentration as shown in Fig. 104.

One peculiarity of the curves observed by both Anderson and Chase has been interpreted by Anderson as indicating reduction of oxidized luciferin during the course of the reaction. If luciferase is added to a luciferin solution that has undergone some spontaneous oxidation, two distinct kinds of luminescence can be observed, rapid and slow, both occurring simultaneously. The rapid luminescence is that ex-

pected from the luciferin in the solution, whereas the slow luminescence represents additional light from continuous reduction of some spontaneously oxidized luciferin which then reoxidizes with light emission. When this reduction and reoxidation occur the curves of total light emission do not reach a plateau but continue upward. This behavior is illustrated in Fig. 105 from the studies of Chase and Giese (1940) on the effect of irradiation of luciferin by ultraviolet light or by visible light in the presence of a sensitizer such as eosin. Under both conditions the spontaneous oxidation of luciferin is accelerated. An exception to this behavior occurs when riboflavin is used as a sensitizer.

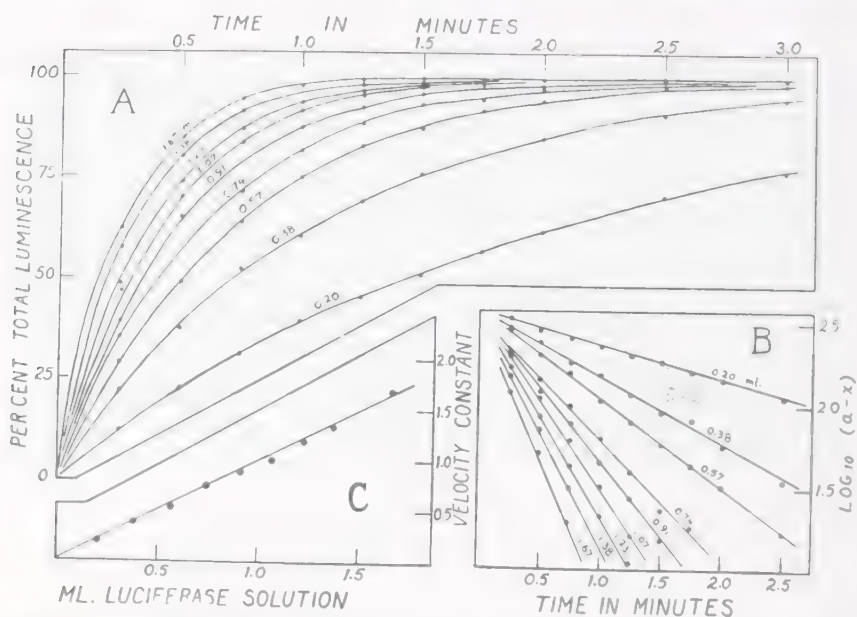


FIG. 104. A, total light emitted (in arbitrary units) *vs.* time in minutes for different luciferase concentrations, indicated in figures on the curves. B, $\log(a-x)$, plotted *vs.* time for different luciferase concentrations. The slope of the straight lines gives the relative velocity constants, which are plotted as a function of luciferase concentration in C. After Chase.

also shown in Fig. 105, where no continuous reduction occurs. Chase has recently found that preliminary extraction of dried Cypridineae with benzene removes some constituent connected with the reduction.

Although carried out on crude luciferin and luciferase solutions, the experiments of Chance, Harvey, Johnson, and Millikan (1940) are of special interest since they are the only ones in which mixing was rapid enough to record the rate of development of light as well as the decay curve. Experiments were carried out in such a way that (1) luciferin and luciferase solutions both containing oxygen could be mixed or (2) luciferin and luciferase could be mixed in absence of oxygen and

the light recorded when oxygen was suddenly admitted. Curves are reproduced in Fig. 106. Luciferase was in high concentration so that the decay curve was often half complete in 0.12 second. The results indicated a time of 0.006 second for the light to rise to one-half maximum intensity in the case of (1) and 0.002 second in the case of (2), three times faster. The experiment shows that luciferin and luciferase combine relatively slowly whereas the combination with

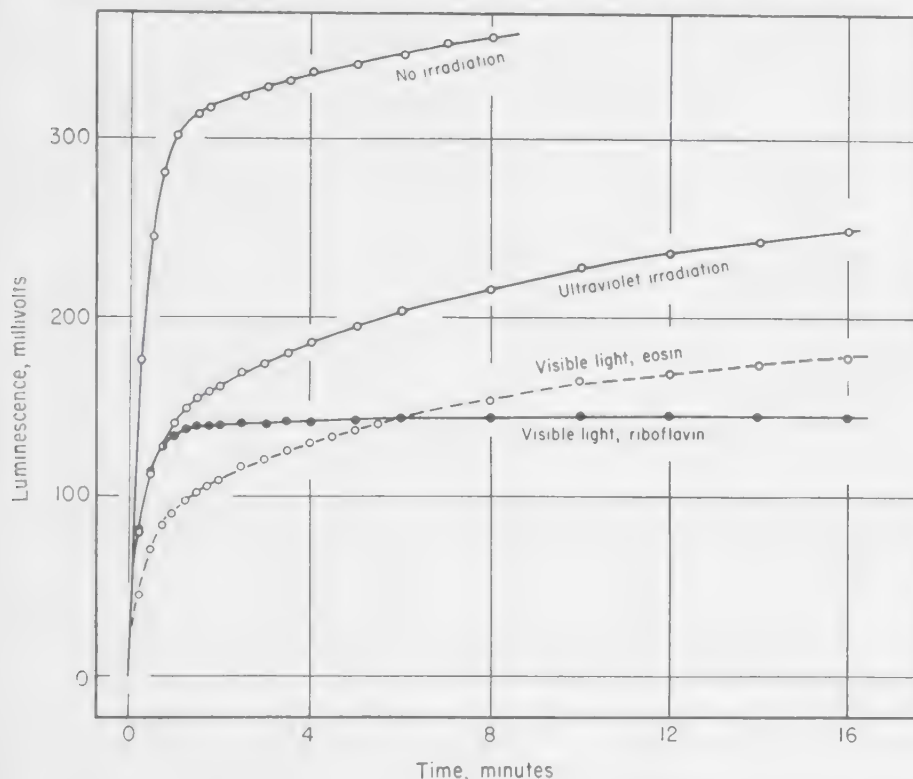


FIG. 105. Total luminescence (in arbitrary units, millivolts) emitted as a function of time on adding luciferase to luciferin solutions which have been irradiated for three minutes with visible light in the presence of about 0.001% concentration of riboflavin. Although an initial bright luminescence occurs, there is no emission after two minutes. The luminescence curves obtained after irradiation of luciferin + eosin with visible light, and after ultraviolet irradiation without a sensitizer, both show not only a bright luminescence during the first two minutes, but also a dim luminescence which persists for at least sixteen minutes. After Chase and Giese.

oxygen is more rapid. Analysis of the reactions led to the following four-step scheme, as a possible luminescence mechanism.

- 1) LH_2 (luciferin) + A (luciferase) \rightarrow A·LH₂ (k_1)
- 2) $\text{A·LH}_2 + \frac{1}{2}\text{O}_2 \rightarrow \text{A·LH}_2\text{·O}$ (k_2)
- 3) $\text{A·LH}_2\text{·O} \rightarrow \text{A}'$ (excited luciferase) + L (oxidized luciferin) + H₂O (k_3)
- 4) $\text{A}' \rightarrow \text{A} + h\nu$ (a quantum of light) (k_4)

Mechanism of Light Emission. The existence of "excited" molecule during chemiluminescence has been considered in connection with bacterial luminescence, and practically every suggestion that has been made for bacterial light could apply to Cypridina luminescence. In the previous diagram luciferase (A) has been designated the emitting molecule, but there is evidence that secondary chemiluminescences in solution are rare and this designation may be incorrect.

It might be argued that luciferin (LH_2) molecules emit the light, since the amount of light produced is proportional to the luciferin

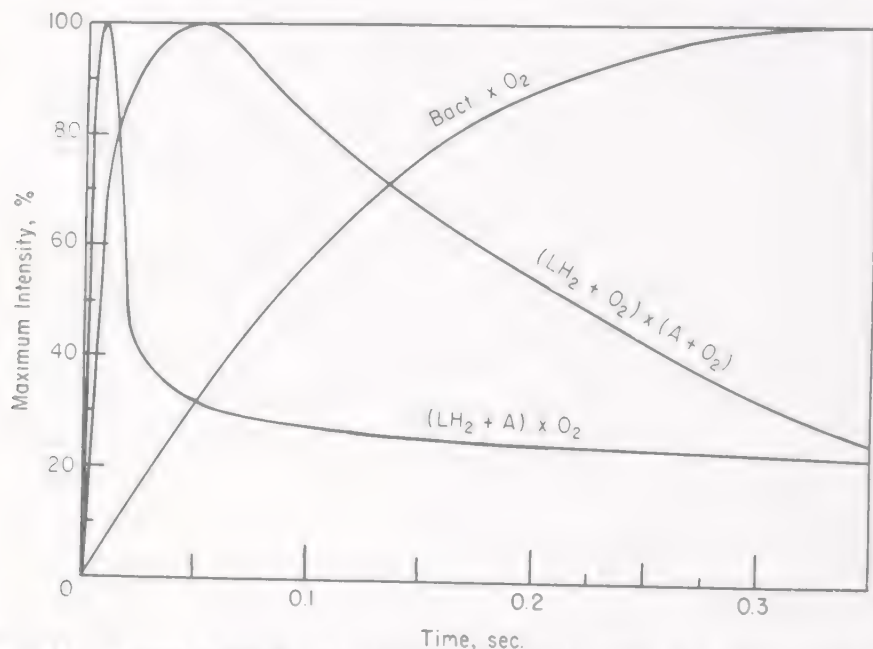


FIG. 106. Curves showing the development and decay of luminescence when oxygen is admitted to luciferin and luciferase previously mixed in absence of oxygen [$(\text{LH}_2 + \text{A}) \times \text{O}_2$]; when luciferin and luciferase both containing oxygen are mixed [$(\text{LH}_2 + \text{O}_2) \times (\text{A} + \text{O}_2)$] and when oxygen is admitted to luminous bacteria without oxygen ($\text{Bact.} \times \text{O}_2$). After Chance, Harvey, Johnson, and Millikan.

present. On the other hand no light appears in absence of luciferase (A). Perhaps, then, the enzyme substrate combination, LH_2A , as suggested by van der Kerk (1942), is the emitting complex. Oxidized luciferin L has also been designated the emitter (Johnson, Eyring, *et al.*, 1945). It is no easy matter to decide from available evidence which molecule acquires the excess energy. Practically all workers on the chemiluminescences have come to different conclusions regarding mechanism of light emission, and designation of the excited molecules in the case of Cypridina must be postponed until more is known of the chemistry of the reactions involved. A discussion of various types

of chemiluminescences and organic fluorescences will be found in the thesis of van der Burg (1943) and in a review article by Anderson (1948). Formulas of some chemiluminescent compounds are shown in Fig. 107.

Effect of Temperature. As in the case of bacteria, Cypridina solutions show increase of light intensity with increase of temperature up to an optimum, above which the light becomes less intense and at a certain maximum temperature disappears. Many workers have noted

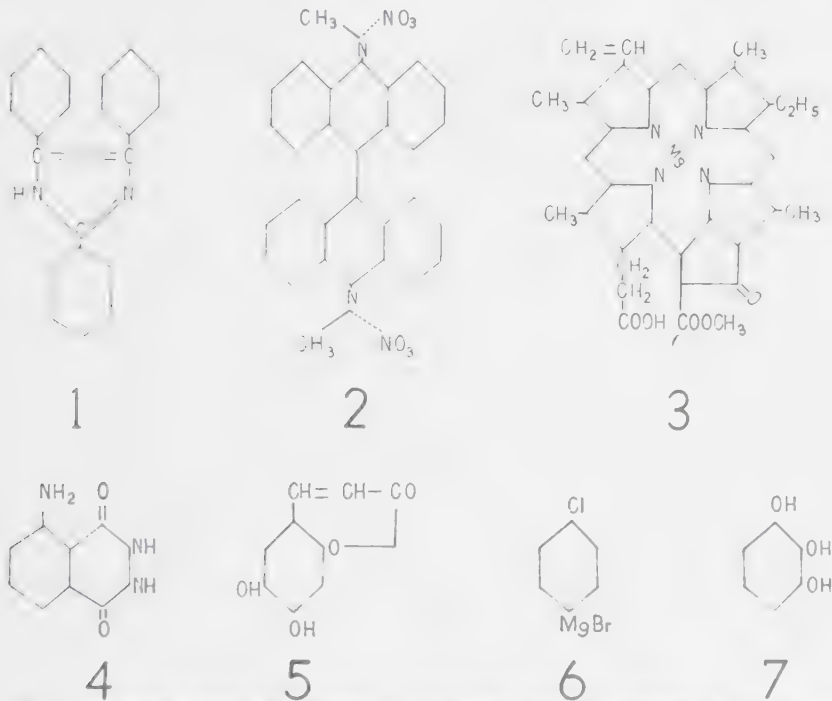


FIG. 107. Various types of organic chemiluminescent compounds. 1, lophin or triphenylglyoxaline; 2, dimethylldiacridinium nitrate; 3, a metal porphyrin compound; 4, luminol or aminophthalhydrazide; 5, aesculetin, present in the glucoside, aesculin; 6, chlor-phenylmagnesium bromide; 7, pyrogallol.

a return of light on cooling. The first observations on temperature effects on ostracods were made by Lund (1911) on *Cypridina squamosa* from Montego Bay, Jamaica. He found that the light disappeared when the luminous secretion in rain water was heated to 50° and returned, if cooled immediately. If heated to 67° and cooled, there was no recovery of light, but when heated to temperatures between 50° and 67°, there was greater recovery the lower the temperature and the shorter the duration of heating.

Harvey (1917) observed a similar behavior of *Cypridina hilgendorffii*, placing the maximum for luminescence at 52–54°, with recovery

on cooling and noting that the more concentrated the mixture of Cypridina luciferin and luciferase the higher the destruction temperature. The normal secretion in sea water permanently lost its luminescence when heated above 70°, but a suspension of dried Cypridina powder could be boiled and cooled with some return of light. It is evident that Cypridina exhibits the reversible and irreversible destruction of luciferase at high temperatures, as well as the effect of temperature on the luminescent reaction itself, which has been studied quantitatively.

Amberson's (1922) original kinetic studies indicated that temperature has a large effect on the light emission. The velocity constants have an average Q_{10} value of 2.74 for 10° temperature intervals between 5° and 35°C. Anderson (1933) had shown that the total light, a measure of non-spontaneously oxidized luciferin, decreases markedly with rise in temperature. This effect is most probably due to increased spontaneous oxidation of luciferin that proceeds without light production at the same time as the light-emitting oxidation. Recently, Chase and Lorenz (1945), in one of the most important kinetic studies, have succeeded in separating the velocity constants of the spontaneous and the luciferase-catalyzed reaction by studying the light emission at five different temperatures between 10° and 35°C.

Assuming that two reactions are occurring simultaneously—one being the luminescent reaction of luciferin and luciferase and the other a non-luminescent oxidation of luciferin—an equation was derived representing two simultaneous first order reactions. This equation has the form,

$$x = \frac{k_1 a}{k_1 + k_2} (1 - e^{-(k_1 + k_2)t})$$

where x is light emitted up to time t , a is concentration of luciferin initially present, k_1 is the velocity constant of the luminescent reaction, and k_2 that of the non-luminescent oxidation of luciferin. The experimentally measured curves are well fitted by this equation.

The variation of k_2 with temperature is described by the Arrhenius equation, which yields a temperature characteristic of about 25,000 calories. When k_1 is plotted against temperature, an optimum for the luminescent reaction appears at about 23°C. with a precipitous decline at temperatures higher than this. An Arrhenius plot of k_1 shows a value for the temperatures characteristic of approximately 5,000 calories on the low temperature side of the optimum. The decrease in the value of k_1 on the high temperature side of the optimum is such that an inactivation of luciferase in a reaction having a temperature characteristic of the order of 50,000 calories is indicated.

The temperature characteristic of 50,000 calories is similar to that for proteins and also similar to that previously mentioned for reversible diminution of the light of luminous bacteria, attributed to reversible denaturation of bacterial luciferase. Chase (1946) has recently demonstrated quantitatively a completely reversible denaturation of *Cypridina* luciferase after a short exposure to 38°C. A later paper (1950) has dealt with the heat inactivation of *Cypridina* luciferase, subjected to temperatures of 40–55° for times up to twenty-four hours. The form of the curve relating luciferase activity to time of exposure is compound in nature and has been analyzed in terms of an irreversible process with rate constant k_1 and a reversible process with constants k_2 and k_3 . The activation energies of k_1 and k_2 were high, about 57,000 calories, indicating protein denaturation, while k_3 was independent of temperature. Inactivation of the enzyme system was dependent on pH, being more rapid at 7.9 and 5.5 than at 6.7.

Effect of Pressure. Preliminary unpublished experiments on the effect of hydrostatic pressure on luminescence of both crude and purified *Cypridina* luciferin² and luciferase mixtures have been made by F. H. Johnson and the author. The effect on luminescence intensity and velocity constant (slope of a log intensity-time plot) were studied at temperatures ranging between 10° and 45°C and pressures of one atmosphere and 5,000 lb/in². Luciferin and luciferase in crude solution behaved differently from the purified mixtures. Under certain conditions, the effect of pressure on purified mixtures was to decrease the light intensity and velocity constant at low temperatures (below the optimum at 23°C) and to increase the already low luminescence intensity and velocity constant at high temperatures, in agreement with the theory postulated in explanation of pressure and temperature effects on luminous bacteria. However, the expected results were not always obtained, and the influence of pressure sometimes varied with length of time after mixing the reactants. It is possible that spontaneous non-luminescent oxidation of luciferin complicates the picture, but it will be necessary to make a very complete analysis of pressure effects to discover and explain the influence of at present unknown variables.

Effect of H-Ion Concentration. Anderson (1933) found the total light emitted depended on the H-ion concentration, decreasing with increasing pH, and Chase (1948) has made an extended study of the effect of pH within the range 5.5 to 8.6 in three different buffer systems — KH_2PO_4 - Na_2HPO_4 , KH_2PO_4 - K_2HPO_4 and NaH_2PO_4 - Na_2HPO_4 —measuring not only total light but also the velocity constant. Attempts to extend the pH range with borate and barbiturate buffers were un-

² Kindly supplied by Dr. A. M. Chase.

successful, because the borate radically altered the luminescent reaction and the barbiturate abolished it.

In confirmation of Anderson, the total light of the reaction was found to decrease with increasing pH to a value at pH 8.6 about 20% of that at pH 5.5. Total light was not influenced by the kind of phosphate buffer used but only by the pH. The effect was not due to non-luminescent oxidation of part of the luciferin during the reaction but apparently to a specific effect of pH upon the light-emitting system, analogous to salt effects described below.

The velocity constant of the reaction, representing the enzyme activity was affected not only by the pH of the buffer but also by its ionic composition. So far as pH was concerned, maximum luciferase activity occurred at about pH 7.2. It decreased to about 50% of the

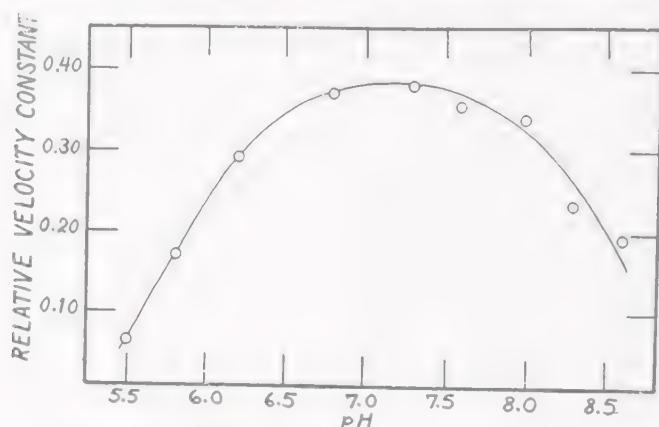


FIG. 108. Variation in luciferase activity with pH. The relative velocity constants have been plotted against the pH of the KH_2PO_4 , Na_2HPO_4 buffers in which the luminescent reactions were measured. Activity is maximal at about pH 7.2. After Chase.

maximum value at pH 8.6 and to about 15% of the maximum value at pH 5.5, as indicated in Fig. 108.

At any given pH studied, the sodium and potassium content of the phosphate buffer also influenced the velocity constant. At pH 6.8, for example, the velocity constant was measurably greater with the K_2HPO_4 , KH_2PO_4 buffer or with the Na_2HPO_4 , NaH_2PO_4 buffer than with the conventional Na_2HPO_4 , KH_2PO_4 buffer. The Na and K ion effect appeared to be upon the enzyme, luciferase, or the enzyme substrate complex, not on the substrate, luciferin.

Effect of Salts. While engaged in the study of methods for the determination of luciferin, Anderson (1937) observed that the total light emitted by the same concentrations of luciferin and luciferase increased with increasing NaCl concentrations and also varied greatly

with the kind of salt present in the solution. If the total light emission with no salt is 1, the emission with various salts in 0.0095 *M* concentration is as follows: NaCl, 2.3; KCl, 2.2; KBr, 2.0; NaBr, 1.9; KF, 1.4; KNO₃, 1.3; K₂ oxalate, 1.2; K₂SO₄, 1.1; KCNS, 0.12; KI, 0.08.

Effect of Drugs and Narcotics. It has long been known that narcotics will reversibly inhibit enzyme activity. The author (1917) determined that the light of Cypridina extracts disappears when ethanol to 20% or butanol to 8% is added to them, and the light returns on dilution. This reversibility is characteristic of a series of alcohols from methyl to hexyl, recently studied by Chase (1951), who showed that the alcohols act on luciferase and that the effect increases with the length of the carbon chain. Each additional carbon atom increases the effectiveness from two to three times, as has been found for the activity of such a series on the narcosis of living organisms.

A similar reversible effect of urethane on luminescence of Cypridina extracts was observed by Taylor (1934). Quantitative measurements by Johnson and Chase (1942) have shown that urethane and various sulfa drugs in proper concentration also reversibly inhibit Cypridina luminescence. The velocity constant of the reaction is decreased without affecting total light emitted. Para-aminobenzoic acid inhibits luminescence, and its effects are partially additive with sulfanilamide. Quinine also decreases the luminescence of crude luciferin-luciferase mixtures, according to Johnson and Schneyer (1944).

Effect of Heavy Water. Anderson and Harvey (1934) determined that in 81% deuterium oxide, the total amount of light emitted by a certain mixture of luciferin and luciferase increased by about 20%, and the rate of the reaction at half completion was about 60% of the same mixture in distilled water.

Physical Characteristics of the Light

All observers agree that the luminescence of Cypridina is blue in color and remarkably bright. When the dry powder is moistened the particles of gland material appear as brilliant specks. Nichols (1924), using a modified optical pyrometer method, has determined that their brightness ranges up to 16 millilamberts.

The first measurements of spectral energy distribution were made by Coblentz and Hughes (1926) whose curve, giving energy as a function of wave length, is reproduced in Fig. 109. It was obtained from a photograph of the Cypridina emission spectrum by measuring the density of the plate in different wave length regions after calibrating the plate by varying exposures to a standard lamp. A second

curve has been obtained by Eymers and van Schouwenburg (1947) again using the photographic method, but calibrating the plate in terms of constant exposure and variable light intensity, which they considered a better procedure. They also plotted the energy as a function of

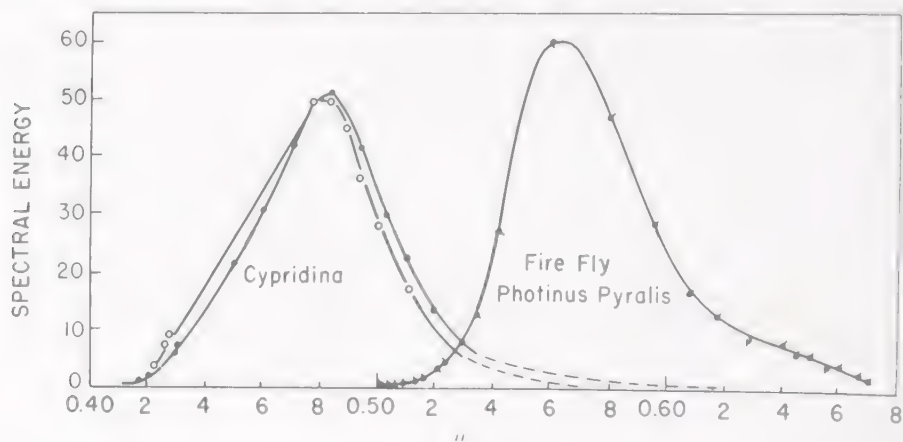


FIG. 109. Spectral energy curve of Cypridina compared with that of the fire-fly. Wave length in micra on horizontal. After Coblenz and Hughes.

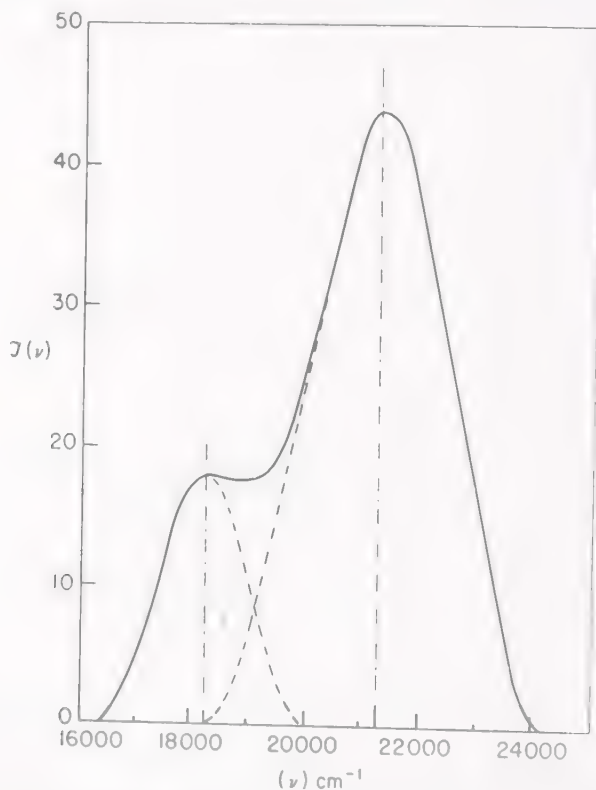


FIG. 110. Spectral energy curve of Cypridina plotted as energy (vertical) vs. frequency (horizontal). After Eymers and van Schouwenburg.

frequency and found certain fundamental frequencies which they thought might represent a common configuration of the emitting molecule. As indicated in Fig. 110, the Cypridina luminescence curve appears to be composed of two frequencies, at $18,200\text{ cm}^{-1}$ and $21,300\text{ cm}^{-1}$, corresponding to wave lengths $549\text{ m}\mu$ and 469μ . However, the work of Spruit (1950) on bacterial spectra and spectra of various chemiluminescent reactions has indicated that such fundamental frequencies do not exist.

COPEPODA

General

The copepods form the largest and most extraordinary order of crustacea. They are all small and live under the most varied conditions, occurring in both fresh and salt water, although chiefly the latter. The majority are free-swimming planktonic forms, but some live in mud on the bottom, others are parasites, and a few commensal. It is not surprising to find luminous species among them. Some ten genera are known to be luminous, all belonging to the suborders Calanoida and Cyclopoida, and all pelagic. Because of the small size of most copepods it is difficult to spot a luminous specimen, and knowledge of luminosity would have been meager without the painstaking studies of Giesbrecht (1895).

One of the larger copepods has been called luminous, not because it is rare or difficult to see but because of the remarkable structure of its chitin, which gives interference colors in the light. This is the genus Sapphirina, one of the striking marine plankton organisms, known to every student of the "tow." Thompson (1831) had described it as luminous and, according to Meyen (1834), the luminous animal called Oniscus fulgens by Anderson in 1747 was a Sapphirina. It is quite certain, however, that this genus is not luminous. Its beautiful opal-like play of colors is only seen by reflected light and disappears in total darkness.

Possibly the first observation of luminous copepods is due to Fabricius in 1780, who identified them as Cyclops brevicornis in his "Fauna Groenlandica." According to Giesbrecht (1895), Fabricius probably saw the modern genus, Metridia, common in Davis Strait, although the record is questionable. One of Viviani's (1805) figures looks like a copepod, and there can be no doubt but that Baird (1830, 31) in his articles on the "Luminousness of the Sea" saw luminous copepods called Cyclops. Later (1843) he figured five species belonging to

the genera *Oithona* and *Cyclopsina* which Giesbrecht has designated species of *Corycaeus*.

Luminous copepods have also been reported by Dana⁶ (1846), Boeck (1865), by Lilljeborg on the snow at Spitzbergen in 1875, Giesbrecht (1892, 95), Dahl (1893, 94), Verhoffen (1895), Sars (1907, 25), Kiernik (1908), and many others. Dahl (1893) correctly described structures in different species of *Pleuromma* as light organs and not eyes and listed (1894) *Pleuromma* and *Metridia* as the only luminous genera of copepods. Vanhöffen saw the greenish luminous secretion of glands of *Metridia longa* from Greenland empty into the sea water, and Kiernik described luminous *Metridia lucens*, *Chiridius obtusifomis*, and *Euchaeta* sp. from Bergen, Norway.



FIG. 111. The luminous copepod, *Oncaea conifera*, male and female in copulo. After Giesbrecht.

Of all these men, the work of Giesbrecht is outstanding. His monograph (1892) on the copepods of the bay of Naples contains a preliminary description of luminous species, together with most carefully drawn and beautifully reproduced figures which illustrate the bizarre forms and gaudy coloring of these minute crustacea, some of which are shown in Figs. 111 and 112. A later paper (1895) dealt specifically with luminescence of copepods in relation to other luminous animals. The five luminous species studied were *Pleuromma abdominale*, *P. gracilis*, *Leuckartia* (now *Lucicutia*) *flavicornis*, *Heterochaeta* (now *Heterorhabdus*) *papilliger*, and *Oncaea conifera*. These forms from Naples showed a seasonal variation, being brightly luminescent during the early months of the year, but in the summer and fall, the light was not seen. Like most animals they luminesce only on stimulation.

Because of the large number of families containing no known lumi

⁶ Dana (1846) stated that in the genus *Corycaeus* there was a structure with two lenses separated by an unobstructed space, which might be a luminous organ. "I was never satisfied that the species was phosphorescent."



FIG. 112. Luminous copepods. Left to right, *Pleuromma gracilis* female; *P. abdominale*, male; *Corycaeus rostratus*, male. After Giesbrecht.

nous species, only the principal suborders of Copepoda and only the luminous families and genera (in italics) are given in the following classification of V. Brehm.

Copepoda

Gymnoplea or *Calanoida* (28 families), including

Aetideidae (*Chiridius*)

Euchaetidae (*Euchaeta*)

?*Phaennidae* (?*Cephalophanes*)

Lucicutiidae (*Lucicutia* = *Leuckartia*)

Metridiidae (*Metridia*, *Pleuromamma* = *Pleuromma*)

Heterorhabdidae (*Heterorhabdus* = *Heterochaeta*)

Pontellidae (*Pontella*)

Podoplea or *Cyclopoida* (43 families), including

Oncaeidae (*Oncaea*)

Corycaeidae (*Corycaeus*)

Sapphirinidae (*Sapphirina*)

Philichthytes (1 family, *Philichthyidae*)

Dichelestia (1 family, *Dichelestiidae*)

Caligi (5 families)

Chondracanthi (1 family, *Chondracanthidae*)

Lernaeae (5 families)

Lernaeopodae (1 family, *Lernaeopodidae*)

Choniostomata (1 family, *Choniostomatidae*)

Herpyllobii (1 family, *Herpyllobiidae*)

Morphology and Histology

The number and distribution of luminous glands in each species is always the same, even during the various later stages of development. Under the microscope Giesbrecht (1895) could observe the green luminescence of material secreted by certain skin glands which do not differ from other skin glands, except in their yellow color. The photogenic cells always contain droplets of a greenish yellow material while the droplets in common skin glands are colorless.

Eighteen rather large pear-shaped luminous glands were found in *Pleuromma abdommale* on various body regions and on the furca in the same position in male and female. *Pleuromma gracile* had seventeen light organs, *Leuckartia flavicornis* had ten, and there were at least thirty-six on *Heterochaeta papilligera*. It is an interesting fact, whose significance is unknown, that sometimes the arrangement is not bilaterally symmetrical. In *Pleuromma* and *Leuckartia* the arrangement of certain of the yellow and colorless glands is in pairs. In *Heterochaeta* they are actually "twinned" and have a common opening. The significance will be discussed under biochemistry. There appeared to be no special muscles to squeeze out the secretion, but Gies-

brecht thought that possibly pressure exerted by the abdominal muscles is involved. He did not observe a nerve supply.

In *Oncaea conifera*, belonging to another suborder, the luminous glands were over seventy in number in the female but fewer in the male. They were larger and of a more irregular form than in *Pontella* and filled with a finely granular mass instead of large clear droplets. Only the body glands were luminous, not those on the limbs. The luminescence was blue like that of euphausiid larvae, rather than green as in the other copepods.

In another copepod, *Cephalophanes*, Sars (1907) had described "deux organes volumineux d'une structure toute singulière" which he thought might be luminous organs, and he later (1925) called the structures light organs. However no observation of light has been made, and Steuer (1928) has regarded them as "eigenartig umgebildete Augen."

Biochemistry

Giesbrecht (1895) carried out quite a few chemical experiments. He found that when copepods were dried on filter paper and later moistened even after a period of three weeks, light would appear. The ability to luminesce was also retained when dehydrated with glycerine, but only for ten hours. Absolute alcohol was ineffective in preserving the light.

In view of the necessity of oxygen for many luminescences, Giesbrecht attempted to test its effect on copepods, using well-boiled water. He found that light appeared under these "oxygen-free" conditions. The method is of little value and the author (1926) has reinvestigated the question, using pure hydrogen to remove the oxygen. The experiment was carried out at Naples in December, 1925, with a mixture of the various copepods studied by Giesbrecht. If these animals are heated slowly in sea water with air they luminesce brightly, but if heated slowly in sea water containing platinized asbestos through which hydrogen has been passed, there is no light. When air is readmitted the solution becomes luminescent. There can be no doubt of the necessity of oxygen.

Using the same mixture of copepods under the microscope, the author tested for fluorescence by directing a beam of ultraviolet light on the animals from above. Under these conditions it is easy to see the greenish blue bioluminescence from the unicellular glands scattered here and there, and when this bioluminescence fades, many, but not all, of the originally luminescent spots are brightly greenish blue fluorescent in ultraviolet light. In fact, it is only by screening off the

ultraviolet that the difference between bioluminescence and fluorescence can be detected. Although Burghause (1914) reported inhibition of copepod luminescence by light, in the author's experience the bioluminescence is not inhibited by sunlight.

It will be recalled that the yellow luminous glands are frequently paired with colorless skin glands and that in some cases they are "twinned," and have a common opening. This might indicate that two substances are necessary for luminescence. Giesbrecht was unaware of Dubois' work on luciferin and luciferase, but he found no evidence that the mutual reaction of luminous glands and skin glands is necessary for light production. Where the two types are well separated, he could observe that light appeared at the mouth of the luminous gland when its secretion could not have come into contact with the secretion of any other skin gland.

The author (1926) made a special effort to demonstrate luciferin and luciferase in copepods in the usual way, but always with negative results. In order to avoid oxidation, copepod luciferin was prepared in absence of oxygen by boiling the animals in a stream of pure hydrogen saturated sea water. When cooled and air was admitted, this solution gave a faint luminescence, indicating considerable resistance to heating, but on mixing with copepod extracts whose luminescence had disappeared (copepod luciferase) or on mixing with *Cypridina* luciferase, the light became no brighter. *Cypridina* luciferin also gave no light with copepod "luciferase." A test of the possible role of adenosine-triphosphate in luminescence of these organism has not been made.

ISOPODA

A few instances of luminous isopods have been reported, mostly known to be due to luminous bacterial infection. It is possible that such an infection may explain the case of *Idotea phosphorea*, concerning which Verrill and Smith⁷ have written, "It is, as its name indicates, decidedly phosphorescent." The animal lives among eel grass and *Fucus* along the New England coast.

In the autumn of 1933 Haneda (private communication) observed one specimen of *Megaligia* among numberless non luminous individuals at Tomioka beach near Yokohama. The light was proved to be a luminous bacterial infection and the bacteria were cultured.

Somewhat later Haneda (1939) observed a wood louse in the Palau Islands, moving on the ground behind the coral museum of the Tropical Biological Station and giving off a bluish white light. The Japanese

⁷A. E. Verrill and S. L. Smith. Report on the invertebrate animals of Vineyard Sound and adjacent waters. Washington, 1874, p. 22.

name is Waraji-mushi, and the animal is very closely related to *Porcellio scaber*. The whole body luminesced, but not the legs, and the light lasted five days. Only one luminous specimen was observed during an eight months' stay. At the same time Haneda saw a piece of pork and a cucumber, all shining from luminous bacteria, probably transferred by the knives used in cutting marine fish. From the wood louse, luminous bacteria similar to those in the sea, were grown on artificial media.

AMPHIPODA

This order of almost exclusively marine crustacea contains about 45 families and over 3,000 known species. They are usually relatively small, with the body flattened laterally, and frequently live on the sea-beach or under stones. Certain common varieties are known as sand fleas. It is quite certain that the luminescence of most amphipods is due to bacterial infection and will be discussed below. However a few species are deep sea dwellers and two of these have been considered self-luminous, although the light of neither of the two has been observed. They possess structures that could be photophores.

Woltereck (1905) has described paired gland-like devices with reflectors on the sides of the head in a genus of the Lanceolidae, *Scypholanceola*. In this form, which was caught by the Valdivia and also the Swedish South polar expeditions at 2,000 meters, the eyes have been reduced to mere pigment spots without optical apparatus. There appears to be no other function for the organ than light production and Woltereck believed that other genera of the Lanceolidae might also be luminous.

A second species of Amphipod, *Streetsia nyctiphanes* nov. sp., belonging to the Oxycephalidae, was caught by the research ship *President Theodore Tissier* off the Atlantic coast of Morocco at 500 meters. This form has been studied by Fage (1934), who described three suspected luminous organs on each side of the body at the sixteenth segment. They were oriented obliquely backward, of elongated cone shape, with the sides and base covered with a brown pigment layer, and within a lining of glandular cells like those of the luminous fish *Cyclothone*. Some six other species of *Streetsia* are known, all bathypelagic, but without the "luminous organs."

The position of *Scypholanceola*, *Streetsia*, and the beach fleas in a classification (luminous genera in italics) of the Amphipoda by J. Reibisch is as follows:

Amphipoda

Gammaridea 143 families including Gammaridae with 60 genera, including Gammarus, and Talitridae with 13 genera, including Talitrus, Orchestia and Hyalea

Ingolfiellidea (Ingolfiellidae, with Ingolfiella)

Laemodipoda (Caprellidae and Cyamidae)

Hyperidea (19 families, including *Lanceolidae* with *Scypholanceola*, *Pradanceola* and *Lanceola*; *Hyperiidæ* with *Hyperia* and 8 other genera and *Ozycephalidae* with 7 genera, including *Streetsia*)

In addition to the possible examples of self-luminous amphipod just cited, there are a number of cases where infection with luminous bacteria results in a brilliantly luminous individual while still living and moving about in a normal manner. The observations of Thulin and Bernard (1786) on a luminous "crevette," identified by Geoffroy as *Cancer macrurus rufescens* and by Ehrenberg (1834) as *Cancer pulex*, from a river near Trans, southern France, may be the first record of such a luminous amphipod. The common fresh water amphipod of Europe is *Gammarus pulex*. The fact that not all the animals observed were luminous is strong evidence in favor of the view that a luminous bacterial infection was involved, similar to that of the fresh water shrimp of Lake Suwa, Japan, described by Yasaki (1927).

Other instances are recorded in the literature. It is very probable that Viviani (1805) was the first to describe luminous marine amphipods. In his "Phosphorescentia maris" there are figured six species of Genoese crustaceans, called *Gammarus*, undoubtedly amphipods, but few systematists have been able definitely to identify them. Stebbing (1888, p. 75), in his detailed discussion of the older literature, has suggested that some might belong to the genus *Hyperia* of the *Hyperiidæ* or *Hyale* of the *Talitridæ*, closely related to *Talitrus* and *Orchestia* which frequently become luminous while alive, due to infection with luminous bacteria.

Tilesius (1819) also figured luminous amphipod crustacea, which Stebbing (1888, p. 108) has suggested might belong to the *Hyperiidæ* and finally, also according to Stebbing (1888, p. 327), Van Vollenhoven (1860) recorded the phosphorescence of *Orchestia littorea*. It has been suggested that luminescence of amphipods might be due to dinoflagellates which they have ingested, and Quatrefages did actually observe *Talitrus* along the shore which were luminous from *Noctiluca* that stuck to the carapace.

However, the more plausible bacterial origin of the light has been demonstrated by the classic work of Giard (1889, 90) and Giard and Billet (1889) on sand fleas. They appear to be the first to establish definite proof of the natural infection of any animal with luminous bacteria, giving rise to a luminescent malady that eventually led to the death of the animal. A single specimen among thousands of the genus, *Talitrus* was found at Wimereux. The whole body and ap-

pendages, even to the tips of the legs and antennae, glowed continuously with a bright greenish luminescence. Under the microscope, numerous bacterial (a *Diplobacterium*, $2\ \mu$ long) were observed among the muscles, which were themselves profoundly altered. Giard inoculated other individuals of *Talitrus* and *Orchestia* with blood from the luminous sample. These also became luminous and in his first experiments six generations of luminous *Talitri* were obtained by repeating this process. "The disease follows a very regular course. At first one sees only a luminous point at the place of the puncture. After the lapse of from forty-eight to sixty hours, the whole animal is phosphorescent, but with a white light which has little external diffusion. At this time the *Talitrus* shows great activity. After the third or fourth day the phosphorescence becomes brilliant and of a fine greenish tint, and the animal throws out a bright light all around it. It may be perceived at a distance of 10 meters. . . . At this phase of the malady the *Talitrus* progresses more slowly; it can still issue from its burrow, which it illuminates, and return there if disturbed. The period of this state may last from three to six days; then comes a period of immobility during which the phosphorescence retains all its brilliancy. Lastly in three or four more days the animal dies; the body remains phosphorescent for some hours and then acquires a characteristic brown tint. Frequently the point of inoculation is surrounded by a small blackish circle."

Passage of the bacterium through another type of sand flea, *Orchestia*, did not change its virulency for *Talitrus*. The amphipod, *Hyale Nilssoni* Rath, and specimens of *Ligia oceanica*, as well as crabs (*Cancer moenas* and *Platyonychus latipes*) were also made luminous by inoculation. Giard (1890) was able to grow the bacteria on salt-containing bouillon, nutrient agar, or gelatin and potatoes, although the colonies were not always luminous. Nevertheless it was possible to inoculate *Talitrus* and *Orchestia* with these non-luminous cultures and produce the luminous malady. During the winter in Paris these cultures became modified, so that they lost the virulence necessary to infect the amphipods, but it was possible to revive the virulence of the bacteria by growing them on fish, after which inoculation of amphipods resulted in luminous animals. The form first isolated is usually referred to as *Bacterium giardi*, but other species of bacteria were used for inoculation and also produced luminous *Talitri*.

No further records of a natural luminous bacterial infection of sand fleas are to be found until 1925 when students at the Marine Biological Laboratory at Woods Hole, Massachusetts, brought several luminous

* From the English account in *Ann. & Mag. Nat. Hist.* (6) 4, 476-78, 1889.

specimens of *Orchestia longicornis* to me. They were found along shore, among piles of dead eel grass. These presented exactly the same picture of massive luminous bacterial infection which Giard described many years ago.

The bacteria were studied by Inman (1927), who was able to grow brilliant luminous colonies on sea water-peptone-agar brought to a pH of 8.1. They remained luminous in artificial culture for over two years. Inman also found that various sand fleas and crustacea could usually but not always be successfully inoculated. A study of the bacterial content of the intestinal tract of normal non luminous sand fleas almost always revealed a few luminous bacteria that could be isolated and grown on artificial culture media.

There is thus always present the possibility of infection of the sand flea, and it is perhaps surprising that such a malady is not more common. However, despite the large number of biologists who come to Woods Hole each summer, no new cases of luminescence have been reported. Inman, himself, examined over 20,000 sand fleas and found only an insignificant number that were luminescent, but once the luminescence has appeared the individuals are doomed; they never recover and death inevitably ensues.

MYSIDACEA

This small group of shrimp-like crustacea contains a few luminous species but little is known of the light production. Almost exclusively marine, the mysids are sometimes considered a suborder of Schizopoda, together with the euphausiids. They contain some 300 species divided among five families. Many early explorers undoubtedly saw these animals, and in later expeditions luminous mysids have also been observed, without much detail regarding species. Thompson (1829) described a luminous mysid as *Cynthia thompsonii*, now known as *Siriella thompsonii*. Baird (1831) also figured a *Siriella*. Gigliotti (1870) declared that phosphorescence of both Atlantic and Pacific oceans was sometimes due to *Mysis*. The *Challenger* naturalists saw luminous mysids and Gadeau de Kerville (1890) mentioned young *Mysis* as one of the luminous forms observed by Edmond Perrier while on the *Talisman* expedition (1880-83).

Gnathophausia zoëa was reported by Chun as luminous when caught at 1,326 meters by the *Valdivia* expedition in 1898. Finally, Annadale observed luminescence of *Gastrosaccus* from India. His description, added to the manuscript of Dr. W. M. Tattersall, who described the specimens obtained, is as follows: "The specimens of *Gastrosaccus simulans*, obtained at Puri, were found at night at the

water's edge on a sandy beach facing the open sea. Their presence was detected in the first instance owing to their brilliant luminosity which was of a general nature."⁹

The position of the above mentioned luminous genera in a classification of the Mysidacea by C. Zimmer can be seen from the following table:

Mysidacea

Lophogastridea

Lophogastridae (Lophogaster, Paralophogaster, Chalaraspis, Ceratolepis, *Gnathophausia*)

Eucopiidae (Eucopia)

Mysidea

Lepidothalmidae (Lepidops = *Lepidophthalmus*, *Spelaeomysis*)

Petalophthalmidae (*Petalophthalmus*, *Hansenomysis*,

Ceratomysis, *Scolophthalmus*)

Mysidae

Boreomysinae (Boreomysis)

Siriellinae (*Siriella*, *Hemisiriella*)

Rhopalophthalminae (*Rhopalophthalmus*)

Gastrosaccinae (5 genera, including *Gastrosaccus*)

Mysinae (57 genera, including *Mysis*)

Mysidellinae (*Mysidella*)

Knowledge of the behavior, physiology, and biochemistry of mysids is almost completely lacking, and histological study has been scanty and not too detailed. The pigmented light organ of *Gnathophausia calcarata* has been studied by Illig (1905). It is situated in a rounded projection of the second maxilla at the base of the exognath. In sections, rows of gland cells which secrete a luminous fluid can be readily seen. The secretion is visible in fixed preparations as whitish threads. It is carried by means of two ducts into a great reservoir whose opening is at the end of the rounded protuberance. Muscles connect with the reservoir and are no doubt instrumental in rapidly forcing the secretion into the sea water. The luminescence has been described as greenish by those who have seen the living animal.

EUPHAUSIACEA OR SCHIZOPODA

General

The euphausiids or schizopods contain shrimp or prawns which differ from another group of shrimp, the decapods, in that the thoracic appendages are all biramose, i.e., their feet are split into two parts.

⁹From p. 159 of W. M. Tattersall, 1915. *Fauna of Chilka Lake*. Mysidacea. *Mem. Indian Mus.*, Vol. V, pp. 149-61. Dr. Tattersall has written me that he observed no special luminous organs on the preserved specimens. The light may have come from infection with luminous bacteria.

They are all pelagic, occurring in great swarms, coming to the surface at night, and migrating to deep water in daytime (see Esterley, 1913, and p. 346). The order consists of a single family, the Euphausiidae, with 11 genera: *Bentheuphausia*, *Thysanopoda*, *Meganycetiophanes*, *Nyctiophanes*, *Euphausia*, *Pseudeuphausia*, *Thysanoessa*, *Tessara brachion*, *Nematoscelis*, *Nematobrachion*, and *Stylocheiron*. All genera except *Bentheuphausia* possess luminous organs and are italicized, although luminescence from all approximately eighty species has not been observed in the living animal.

Dr. W. M. Tattersall, an authority on this group, has prepared for me an interpretation of the early reports of luminescence of euphausians, for it is almost impossible to recognize the species. The names *Cancer*, *Gammarus*, or *Oniscus* indicated some sort of shrimp-like crustacean, euphausiid or decapod, and were described as luminous as early as 1747 by Anderson. Macartney's (1810) figure of *Cancer fulgens* is regarded as an euphausian by Tattersall as well as the luminous *Nocticula* (sometimes called *Noctiluca*) *banksii* of Thompson (1829) and the luminous crustacea of Baird (1831), Westwood (1831), Dana (1852), and M. Sars (1864).

The *Challenger* expedition caught many luminous Euphausians in 1880, and Thomson and Murray (1885) in the Narrative¹⁰ wrote that they frequently observed the living animals, which had "a pair of bright phosphorescent spots directly behind the eyes, two other pairs on the trunk and four other spots situated along the median line of the tail." The eye spots were large and gave a most brilliant and bluish white light. After the first flash, there persisted a dull glow. Subsequent flashes were less and less bright, and finally the animal lost its ability to luminesce. Under the microscope the organs appear as "pale red spots with a central clear lenticular body." One of the euphausians is shown in Fig. 113.

G. O. Sars (1885) in his report on the Schizopoda for the *Challenger* expedition devoted three pages to what he called their "luminous globules," which Claus (1863) had designated accessory eyes. Sars wrote:

"Notwithstanding this great resemblance to visual organs, researches conducted with the living animal have convinced me that none of these organs are coadjutory to sight, but that they all together constitute a very complicated and peculiarly developed luminous or phosphorescent apparatus. The chief light producing matter I have found to be the fibrous fascicle lying in the centre of the globular corpuscle. Even if the organ be crushed, and this fascicle extracted, it

¹⁰ Vol. I, part 2, p. 743.

still continues to give forth a comparatively strong phosphorescent light when seen in the dark. The lenticular corpuscle placed just in front of this fibrous matter may, I conceive, act as a condenser, producing a bright flash of light, the direction of which admits of being changed at the will of the animal, by simply rolling the organ by means of its muscular apparatus. The pigment-coating of the hinder portion, and the diaphanous condition of the front part, may likewise be easily explained as subservient to such a function."

In addition to Claus, Patten (1886) also described the organs as accessory eyes, rejecting Sars' (1885) interpretation as luminous organs



FIG. 113. The shrimp, *Euphausia pellucida*. After Sars.

in the *Challenger* report. Vallentin and Cunningham (1888) who called the light organs, "photospheria," made a careful study of the histology, together with observations on the living animals. Chun (1886), Giesbrecht (1896), Doflein (1906), Trojan (1907), Kiernik (1908), Dahlgren (1916), and others have also observed the light or studied the structure, and there is no doubt of their true function. In many species the photospheres are ten in number, as observed on the *Challenger*, but in *Stylocheiron* there are only three photospheres.

The best account of the behavior has been given by Vallentin and Cunningham who stated that in an aquarium *Nyctiphanes norvegica* gave out short flashes of light from time to time as they swam restlessly back and forth in incessant activity. "When an animal was caught and removed from the water between the finger and thumb,

all the organs emitted a brilliant light for five to ten seconds, while the creature was flapping its abdomen vigorously and trying to escape. Then followed an interrupted series of flashes lasting ten seconds more, and then the animal would become quiet and no light could be seen. But when slight pressure was administered, all the organs flashed again, the duration of the flash being longer when the pinch was stronger. After a day in captivity the luminous response to stimulation became less marked. Except for the fact that light organs are under nerve control, nothing is known of their physiology and biochemical information is completely lacking. Luminescence is intracellular.

Histology

Most workers (Vallentin and Cunningham, 1888; Chun, 1896; Trojan, 1907; Dahlgren, 1916; Pierantoni, 1924) who have studied the histology of the photospheres agree on essential structure but have differed in their interpretation of the region from which the light comes. Eye-stalk organs are somewhat different from the body organs but both types are made up of a pigment sheath, a reflector, layers of cells, a rod bundle or fibrillar mass (*Stäbchenbündel* or *Streifenkörper*) and the lamellae (*Lamellenring*), called by Trojan an external reflector. The latter is a series of rods or plates forming a circular collar or fibrous ring near the outer side of the photosphere. The body organs have in addition to the above structures a well-formed lens, not made up of the external thickening of the cuticle but internal, like the vertebrate eye lens. These features are shown in Fig. 114.

Vallentin and Cunningham endeavored to determine the source of the light by crushing the organ under the microscope. The red pigment was dispersed by the treatment, and all parts of the organ appeared transparent by daylight except the inner surface of the reflector, which was rosy purple by transmitted and yellowish green by reflected light. Evidently a fluorescent substance was present. As time went on, the rosy purple changed to blue and the yellowish green to yellow. In total darkness a luminous spot could be seen in the preparation which proved to be the inner surface of the reflector and not the rod bundle or the lamellar ring.

Sars (1885) and Chun (1896) thought the rod bundle was the source of the light, and Giesbrecht (1896) endeavored to solve the question by studying young larvae of *Euphausia*. He found that their photospheres produce light before the reflector is developed and consequently designated the larval rod bundle (*Stäbchenbündel*) or the *Streifenkörper* of the adult which develops from the rod bundle, as the source of the light.

Giesbrecht argued against the cell layer of the photospheres as the

emitting layer, but Trojan (1907) and Dahlgren (1916) have both designated these cells as photogenic. This view is undoubtedly correct, as it agrees with the situation in the photophores of decapods. Trojan regarded most of the structures of the photospheres as optical accessories, the lens, the refractor (Streifenkörper), the external reflector (Lammelenring), and the principal or internal reflector.

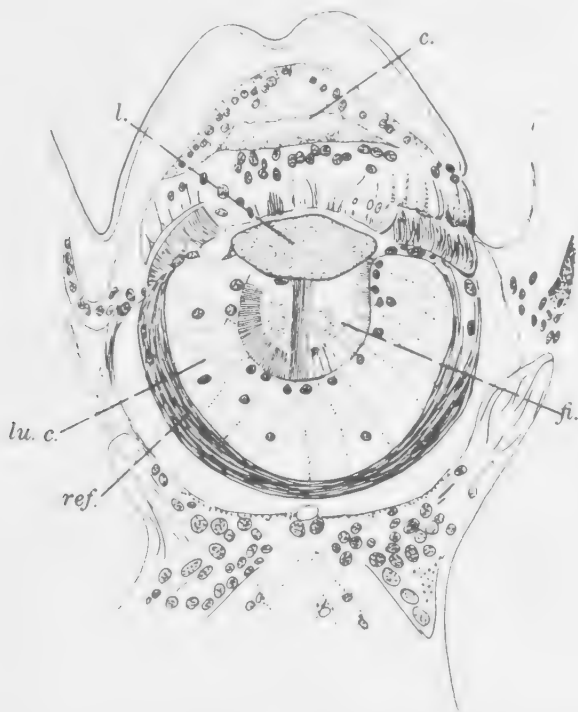


FIG. 114. Section of a photophore of the euphausiid, *Nyctiphanes norvegica*, showing luminous cells, lu. c.; reflector, ref.; rod mass, fi.; lens, l.; blood sinus, c. After Vallentin and Cunningham.

Pierantoni (1921) has made a comprehensive study of the development of the photophores of *Euphausia* but has given another interpretation of the origin of the light. He concluded that the luminescence arises between reflector and lens and is due to symbiotic luminous bacteria. Of the four stages of development, nauplius, calyptopis, furcilia, and adult, the calyptopis stage is especially luminous.

DECAPODA

General

This great order of crustacea contains the shrimp and prawns, cray fish, lobsters, and crabs, some 8,000 species in all. Among these a number of pelagic shrimp are luminous. They live mostly at middle depths and migrate upward at night as do the Schizopods. This ver-

tical distribution and migration in both schizopods and decapods have been studied in recent years by Leavitt (1935, 1938), Chace and Nunnemacher (1937), Welch, Chace, and Nunnemacher (1937), Waterman, Nunnemacher, Chace, and Clark (1939), Moore (1950), and others.

Some species of decapods possess photophores, some secrete a luminous liquid and some possess both types of organ. Probably the first observation of a luminous decapod¹¹ is due to Dana (1852) who described *Regulus lucidus*, now *Thalassocaris lucida*, as "very brilliantly phosphorescent." Another luminous decapod, *Acantheephyra pellucida* [*Oplophorus grimaldii*], from a depth of 500 meters, was caught by Perrier¹² on the *Talisman* expedition in 1880-83. The light came from a large number of photophores scattered on various parts of the body. Coutière (1905) has described the distribution in *Hoplophorus*, *Systellaspis*, and *Acantheephyra*.

However, the most remarkable of these shrimp are the forms which project luminous clouds into the sea water. Alcock (1902) has described the phenomenon in three different species, observed in Indian Seas on the survey ship *Investigator*: "Far the most brilliant of them was *Heterocarpus alfonsi*, both sexes of which poured out, apparently from the orifices of the 'green glands' at the base of the antennae, copious clouds of a ghostly blue light of sufficient intensity to illuminate a bucket of sea water so that all its contents were visible in the clearest detail. *Aristaeus coruscans* [*Plesiopenaeus coruscans*], of which only one specimen, a female, was obtained, also emitted from the same place similar clouds of light, which, however, were less abundant and less brilliant, than those of *Heterocarpus*. The green glands correspond in function with kidneys, so that if, as seems to be the case, the excreta of the prawns are luminous, the fact is by no means an isolated one. The light displayed by the female of *Pentacheles* [*Polycheles*] phosphorus was a steady glow at the points near the openings of the oviducts, where there was found to be a greasy, glandular patch, very much like that seen in the same place in the females of several Indian prawns of the genus, *Peneus*." Lloyd (1907) described luminous *Heterocarpus alfonsi* again and also *Pandalus alcocki*, with a less brilliant light. Since these observations, many naturalists have seen the luminous secretion of deep sea shrimp. W. W. Welch¹³ described the

¹¹ The author expresses deep appreciation to Dr. Waldo Schmitt of the U.S. National Museum for this reference and for much assistance in identification of decapod crustacea mentioned in the older literature.

¹² According to Gadeau de Kerville (1890, p. 89).

¹³ Quoted from Dahlgren (1916). Most observers describe the luminous puff as cloud-like.

discharge as "sometimes coming forth as two rings like the rings blown by a man smoking. These rings moved through the water with their vortex-momentum and ended by sticking against the sides of the glass vessel, still ring-like, where they remained luminous for some time."

Although the eyes of many shrimp appear luminous by reflection of light, Kiernik (1906) has described the eyes of *Pasiphaea etarda* as becoming luminous on chemical stimulation, a report which should be confirmed. There is no doubt of actual luminescence from the photophores of shrimp, which belong to a number of families, although luminosity has frequently been inferred from structure as in the case of *Chlorotocoides* and *Thalassocaris* (Kemp, 1925). There is also a possibility that certain organs in the tail of *Ascetes* are luminous (Okada, 1928).

Burkenroad (1936) considered that compound photophores are restricted to the Penaeidae, Sergestidae, Acantheephyridae and Pandalidae. Welch and Chace (1937, 38) from a study of the eyes of species of Acantheephyra, Systellaspis, and Sergestes have concluded that there is a direct correlation between eye size and presence of photophores, a finding that agrees with the speculation that photophores are used for recognition signals in these deep sea shrimp. The author (1931) never saw light come from the photophores of *Systellaspis debilis* at Bermuda, although the activity of the luminous secretory glands was marked. The experience of Dr. Demmel,¹⁴ who has also studied shrimp in Bermuda, was the same, the luminous secretion is readily excited but the skin organs are rarely luminescent. Chace (1940), in reviewing luminescence of Bermuda caridean shrimp, named *Leptochela bermudiensis*, *Systellaspis debilis*, *Oplophorus spinicauda*, and *O. grimaldii* as luminous. There were no photophores in preserved specimens of *Leptochela*, but photophores quickly disappear in alcohol and Chace wrote, "to my knowledge the photophores of captured specimens of *Systellaspis debilis* have never been seen to emit light." Burkenroad (1936) has also stated that the open (secretory) and closed (photophore) system have never been observed to function in the same species at the same time.

If the purpose of photophores is sex attraction, it is understandable that they might only be functional at certain seasons of the year, and observation of their luminosity would be a matter of chance. Although Gordon (1935) was surprised to find very slight differences in pattern of photophores on the ventral surface of *Sergestes prehensilis*, *S. lucens*, and *S. Challengeri*, Dr. Demmel has assured me that the photophores do have a different distribution in different species but

¹⁴ In conversation with the author.

are exactly alike in the male and female. The extrusion of a luminous secretion may perhaps be considered a device for frightening away predacious animals.

Finally the Japanese fresh water shrimp, *Xiphocaridina compressa*, regularly becomes infected with luminous bacteria. Luminous individuals are found in summer time in Lake Suwa, 100 miles from the sea and 1000 meters above sea level. Yasaki (1927) has described the light as very bright, coming from all parts of the body, even tentacles and legs. Other species of living shrimp and the larva of *Cybister japonica* could be made to luminesce by injection of the bacterium, but dead shrimp infected with the bacteria gave no light. The bacterium is similar to the cholera *Vibrio*, has been isolated by Yasaki and called *Microspira phosphoreum*.

The position of the above luminous genera (in italics) in a classification of the decapoda by H. Balss is as follows:

Decapoda

Natantia

Penaeidea

- Penaeidae* (*Cerataspis*, *Hymenopeneus*, *Aristeus*, *Aristeomorpha*, *Benthescymus*, *Amalopenaeus*, *Gennadas*, *Plesiopenaeus*, *Haliporus*, *Penaeopsis*, *Penaeus*, *Solenocera*, *Funchalia*, *Parapenaeus*, *Parapenaeopsis*, *Sicyonia* and others) pelagic, deep sea, benthon
- Sergestidae* (*Amphion*, *Amphionides*, *Sergestes*, *Acetes*, *Sicyonella*, *Lucifer*, and others) pelagic

Eucyphidea

- Pasiphaeidae* (*Leptochela*, ?*Pasiphaea*, *Psathyrocaris*, *Parapasiphaë* and others) pelagic, litoral, and deep sea
- Bresiliidae* (*Bresilia*) bathypelagic
- Stylodactylidae* (*Stylodactylus*) deep sea
- Atyidae* (*Atya*, *Atyaephyra*, *Caridina*, *Xiphocaris*, *Paratya* = *Xiphocaridina*, *Troglocaris*, and others) fresh water
- Hoplophoridae* or *Acantheephyridae* (*Acantheephyra*, *Hoplophorus* = *Oplophorus*, *Hymenodora*, *Notostomus*, *Systellaspis* and others) benthon and deep sea
- Nematocarcinidae* (*Nematocarcinus*) deep sea
- Campylonotidae* (*Campylonotus*, *Bathypalaemonella*)
- Disciidae* (*Discias*)
- Thalassocaridae* (*Thalassocaris*)
- Pandalidae* (*Chlorotocus*, *Chlorotocella*, *Chlorotoicoides*, *Dorodotes*, *Heterocarpus*, *Pandalina*, *Pandalus*, *Plesionika*, *Parapandalus*, *Pantomus* and others) benthon, litoral, and deep sea
- Psolidopodidae* (*Psolidopus*) deep sea
- Alpheidae* (*Alpheus* and others) litoral in coral reef regions
- Hippolytidae* (*Hippolyte* and others) mostly litoral, many in cold seas
- Rhynchocinetidae* (*Rhynchocinetes*) litoral, marine
- Palaemonidae* (*Desmocarid*, *Palaemon*, *Thyphlocaris*, *Pontonia*, *Anchistus*, *Coralliocaris* and others) fresh water and marine commensal

- Anchistioididae (Anchistioides) marine
 Gnathophyllidae (Gnathophyllum, Phyllognathia, Hymenocera)
 Processidae (Processa = Nika, Nicoides) litoral
 Glyphocrangonidae (Glyphocrangon) deep sea
 Crangonidae (Aegeon, Crangon, and others) benthon, cold seas

Stenopidea

- Stenopidae (Engystenopus, Spongicola, Stenopus, Richardia) marine, deep sea, and commensal

Reptantia

Palinura

- Eryonidae (*Polycheles* = *Pentacheles*, *Willemoesia*) deep sea
 Palinuridae (Palinurus, Panulirus, Jasus, and others) litoral and deep sea
 Scyllaridae (Ibacus, Scyllarides, Thenus, and others) litoral
 Astacura (Nephropsidae = Homaridae, Potamobiidae, Parastacidae) lobsters
 Anomura (15 families) Hippa and others
 Brachyura (27 families) crabs

Histology

The only histological study of the gland (of an unknown species) which secretes luminous material into the sea water, is that of Dahl-

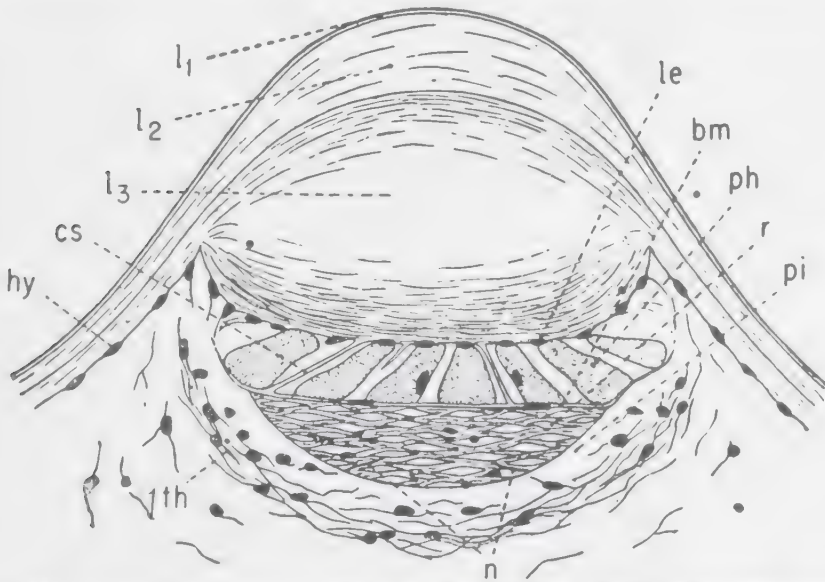


FIG. 115. Section of a photophore of the decapod shrimp, *Sergestes prehensilis*, showing the lens layers, l_1 , l_2 , l_3 ; photogenic cells, *ph*; reflector, *r*; pigment, *pi*. After Terao.

gren (1916), whose drawing shows a rosette of three to seven cells with a duct through which secretion is passed out. The ducts of many of these rosettes form a bundle which opens by pores through the chitin. Four types of gland cells can be recognized, but their significance is uncertain.

Photophores have been studied by Hansen (1903), Kemp (1910), Terao (1917), Okada (1928), Ramadan (1938), and Dennell (1940, 42). Hansen found about 150 luminous organs on every conceivable part of *Sergestes challengerii*. His figure shows the essential eye-like structure of the organ with lens, photogenic cells, reflector, and pigment mantle. Kemp studied *Sergestes challengerii*, *Acantheephyra*

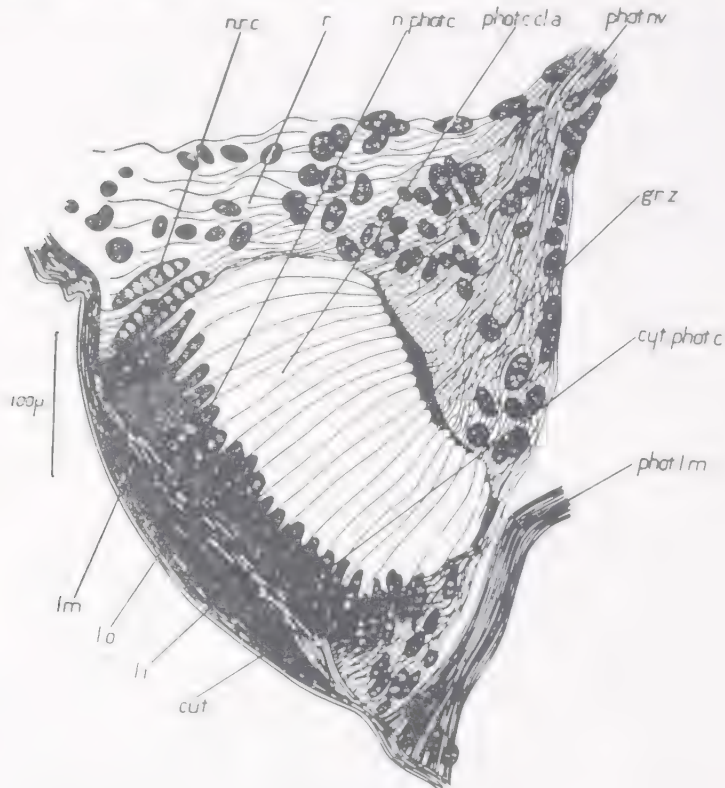


FIG. 116. Section of the pleopod photophore of *Systellaspis affinis*. cut., cuticle; cyt. photo.c., cytoplasm of photogenic cell; gr.z., granular zone; li., inner layer of lens; lm., middle layer of lens; lo., outer layer of lens; n.phot.c., nucleus of photogenic cell; n.r.c., nucleus of reflector cell; phot.cla., clear area of photogenic cell; phot.l.m., longitudinal muscle of photophore; phot.nv., photophore nerve; r., reflector. After Dennell, from *Discovery Reports*.

Systellaspis debilis, and a species of *Hoplophorus*, and Terao published in detail on *Sergestes prehensilis*. His drawing is reproduced as Fig. 115. The lens is made up of a thickening of the three layers of the general cuticle, with a lens hypodermis below. Then comes the layer of large granular photogenic cells with hemal spaces between them. The photogenic cells readily disintegrate when the animals are kept in captivity. Underneath is a basement membrane and a reflector layer, not present in all photophores, which may be modified connective tissue and outside of these tissues is the pigment mantle, as

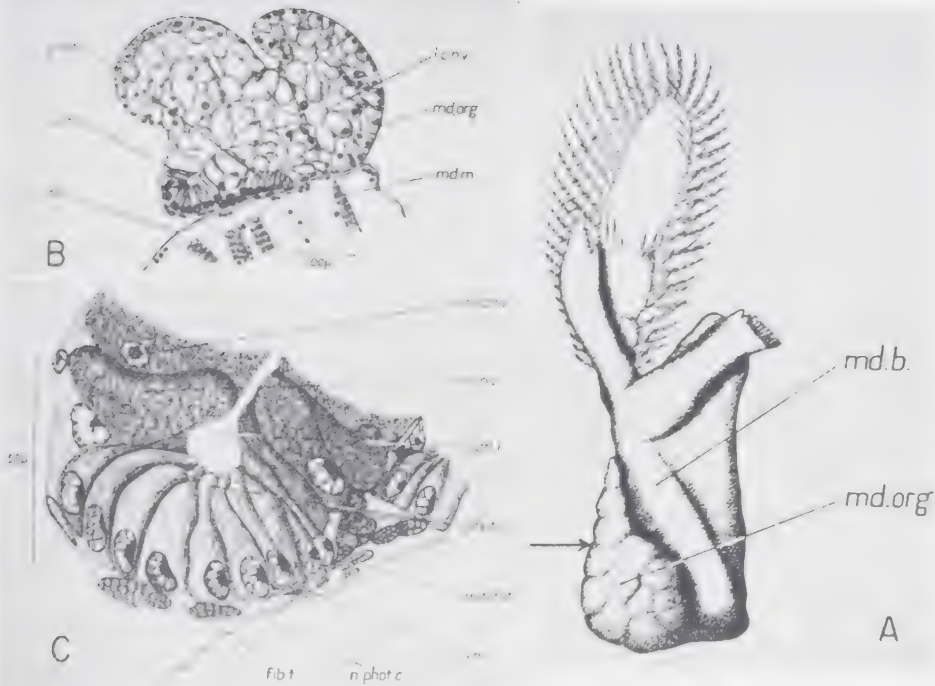


FIG. 117. The mandibular luminous organ of the decapod shrimp, *Parapandulus richardi*. A, mandible (mdb.) with organ (md.org.) at its base (md.b.); B, mandibular organ section showing photogenic cells (phot.c.) near the liver tubules, whose lumen (lum.l.) is indicated; l.c.n.v. is a non-vacuolated liver cell. C, enlarged section of part of liver with photogenic cells. After Dennell, from the *Discovery Reports*.



FIG. 118. Transverse section through the photophore in the roof of the branchial chamber of *Sergestes corniculum*. chit.l., chitinous lens; c.t., connective tissue; n.chit.ep., nucleus of chitogenous epithelium; n.phot.c., nucleus of photogenic cell; phot.c., photogenic cell. After Dennell, from *Discovery Reports*.

if to shield the inner tissues of the shrimp from the light. The whole photophore is surrounded with a theca of connective tissue and is undoubtedly innervated, as Terao observed nerve fragments penetrating the reflector layer.

Histological studies of Okada indicate that certain tail structures of *Acetes*, situated on the uropods, might be closed luminous organs, but they are very different in structure from those of *Sergestes*, and no one has observed light in this genus. The allied genus, *Lucifer*, also possesses a structure of possibly luminous function in the telson, which Burkenroad (1937) has described as a mass of cells surrounded by chromatophores. Despite the name, no one has seen the light of *Lucifer*. The more recent paper of Ramadan is concerned with histology of the photophores of *Hymenopenaeus debilis*, which were described by Burkenroad (1936). A review of the above studies will be found in the article on "Leuchtorgan" of decapods by Balss (1944).

The outstanding work on shrimp photophores based on material collected by the *Discovery* expedition, is that of Dennell, who has observed at least five different types. In addition to the "eye-like" photophores with lenses previously described and shown in Fig. 116, the following groups of luminous cells with no lens system whatever can be distinguished.

1. Superficial photophores of *Sergestes regalis*, consisting of horizontal sheets of interwoven fibers among which are scattered photogenic units. No nerve supply was discovered.

2. Organs of Pesta (luminous liver tubules) suspected of producing light by their discoverer, Pesta, and by Burkenroad (1937) in a later study. They are internal cephalothoracic latero-ventral organs present in many species of *Sergestes*, whose light Dennell¹⁵ has recently observed in Bermuda. They form part of the liver which empties into the alimentary canal. Photophores shown in Fig. 117, made up of groups of modified liver tubules are also present in *Parapandalus richardi*. Their structure is different from those in *Sergestes*.

3. Linear photophores on the roof of the branchial chamber in many species of *Sergestes*, with no lens or accessory structures, shown in Fig. 118.

4. Photophores on the limbs of *Hoplophorus novae zelandiae*, made up of clusters of radially segmented granular masses, apparently devoid of nuclei.

Physiology

Nothing is known of the reflex pathways involved in stimulation of luminescence in shrimp. Some species respond readily to a disturbance of any kind, but even strong stimulation will not produce light in photophores of certain species. It has been already suggested that luminescence may be connected with sexual maturity and only

¹⁵ Private communication.

appear at certain seasons of the year. Another possibility is hormone control of the light organ as has been observed by Greene and Greene (1924) and Harvey (1931) in fish, where injection of adrenaline sets all photophores into activity. However, the author in 1931 tried injecting adrenaline into deep sea shrimp with unlighted photophores, but no light appeared and Dr. Dennell has assured me in conversation that he also could not excite *Systellaspis* to luminesce by adrenaline. Nevertheless other endocrines should be tested. Hormones play an all important part in control of the chromatophores of crustacea in general, and the chances are good that photophores may prove to be similarly controlled if the proper hormone is found.

Terao (1917) has collected specimens of *Sergestes prehensilis*, which has over 150 photophores, and observed the luminescence under natural conditions. He wrote: "in the freshly obtained specimens, the photophores emitted dim greenish yellow light in an intermittent way, each time starting suddenly and vanishing with as much promptitude after a longer or shorter period of illumination. Frequently, after dark intervals of varying length, the lighting up of different photophores in the same body occurred one after another in serial succession, beginning with those at the head end and thence progressing posteriorly, to finish up at the tail end. Each single photophore lighted up for nearly a moment only, and as soon as a light disappeared, another appeared a short distance behind in rapid succession, so that there were scarcely ever observed more than one light alive at a time. It took one to two seconds from start to finish of a single series of illumination of the above sort. At other times, only a limited number of photophores in a certain body region were observed to light up simultaneously, this time the lights remaining steadily alive for several seconds. Most frequently it was the photophores in the neighborhood of the eyes that showed this sort of activity; less frequently those of the third or of the sixth abdominal somite." *Sergestes* fatigues readily, and, even on crushing, the photophore produces much less light than do euphausiids under the same conditions.

Biochemistry

The only work on chemistry of light production among decapods is that of the author (1931), who tested for luciferin and luciferase in the secretion of *Systellaspis debilis*¹⁶ from Bermuda. When brought to

¹⁶ The author made these experiments while a guest at Dr. Wm. Beebe's laboratory on Nonesuch Island, Bermuda. Both luminous shrimp and non luminous ones were obtained, and both were preserved for identification. The bottle marked "luminous"¹⁶ contained shrimp which were identified as *Acantheephyra purpurea* and

the surface and kept in iced sea water. *Systellaspis* lives several hours and the water remains aglow with the luminescent secretion.

To prepare luciferase the animal was placed in a small amount of rain water where the copious secretion forms a luminous liquid whose light disappears after some time. To prepare luciferin the animal was killed in boiling water and ground in a mortar. When the two solutions were mixed, light appeared, indicating the presence of luciferin and luciferase. As the color of the light and the secretory behavior of the shrimp are so similar to *Cypridina*, it was expected that the photogenic substances would interact with those of *Cypridina*, but such was not the case. No light appeared when *Cypridina* luciferase was mixed with *Systellaspis* luciferin or vice versa. Material was not sufficient to study many other properties, but it was noted that adding fresh water or chloroform to the glowing secretion in sea water extinguished the light immediately.

that marked "non-luminous" as *Systellaspis debilis*. It is probable that the bottles were mixed in labelling and that the luminous form called *Acanthephyra* in my book, *Living Light*, was actually *Systellaspis debilis*. The genus *Systellaspis* was originally a part of the genus *Acanthephyra*. It has recently been separated and contains the luminous species.

CHAPTER XI

Arachnoidea and Myriapoda

For convenience of treatment the two above groups will be considered together, although the old group of Myriapoda is made up of rather diverse forms with one order (Chilopoda) closely related to insects.

ARACHNOIDEA

The Arachnoidea contains three sub-classes, the Xiphosura or Merostomata, king crabs or horseshoe crabs, with one genus, *Limulus*, not luminous; the Arachnida or spider-like animals, with eight orders and a few reported luminous species; and the Pycnogonida or sea spiders with one reported luminous species. Very little is known of light emission, as the isolated observations have not been followed up.

The earliest record of luminescence has to do with a sea spider and later reports with land spiders belonging to the order Araneae. The Pycnogonida, containing some ten families, 45 genera, and over 500 species, are exclusively marine, and luminescent forms are to be expected. The Araneae make up a huge group with 62 families and some 20,000 species. If self-luminosity occurs among true spiders, it must be very rare. The relationships are indicated in the following classification, in part from H. S. Pratt and in part from W. Schimkevitch. Luminous groups are in italics.

Arachnoidea

Xiphosura (*Limulus*)

?*Arachnida*

Scorpionida (scorpions)

Palpigradi (*Koenenia*)

Pedipalpi (whip-scorpions)

Solpugida (solpugids)

Chelonethida (pseudo-scorpions)

Phalangida (daddy long-legs)

?*Araneae* (spiders)

Acarina (mites)

Pycnogonida or Pantopoda (Sea spiders)

Nymphonidae (6 genera)

Pallenidae (13 genera)

Phoxichilidiidae (7 genera)

Phoxichilidae (Phoxichilus)

Declopodidae (Declopoda)

Ammothenidae (11 genera)

Oorhynchidae (3 genera)

Tanystylidae (6 genera)

Colossendeidae (*Colossendeis*, Rhopalorynchus, Lecythorhynchus, Pipetta, Pasithoë, Endeis)

Pycnogonidae (Pycnogonium, Pentapycnon)

Araneae

Probably the first record of luminous spiders is to be found in Gilchrist's (1919) paper on luminous earthworms. A friend, Mr. Purcell, told Gilchrist of finding a spider in the grounds of the South African Museum on which luminous patches were plainly visible. "These patches were readily brushed off and were believed to have been caused by contact with some luminous animal, probably a worm."

Another record comes from an observation of Brown (1925) who saw a "ball of light as large as one's thumb" on a trail in Burma, between the villages of Kyawdaw and Thitkydaing, about 120 miles west of Mandalay. On striking a match the continuous light of the same color as that of fire-flies was seen to come from the abdomen of a spider which unfortunately got away when the attempt was made to capture it. A second report has been made by Mr. C. H. Bompas (Brown, 1926) who saw a luminous spider at Shillong in Assam, about 100 miles from the locality in Burma.

There are four possible explanations of spider luminescence: (1) contact with an earthworm or a centipede which secretes abundant luminous material, (2) eating of fire-flies, (3) infection with luminous bacteria or fungi, (4) possession of a true luminous organ. An example of the first explanation has already been given. The second is unlikely, since spiders suck their food rather than eat it. It is doubtful if the luminous material would emit light if reduced to the point where it could be sucked through narrow mouth parts. Spiders often catch fire flies in their webs and inject a poison that causes scintillation of the luminous organ, but there are no records of luminous spiders which might have resulted from eating the fire-fly. Moreover, the whole digestive tract might be expected to show luminescence if the light comes from a fire fly meal, but Brown observed that only the abdomen was luminous, not the thorax.

In this connection the author cannot resist relating his own experience with a luminous toad. While collecting fire flies in Cuba, the

toad was noticed hopping about a field near Santiago de las Vegas early in the evening. A closer examination showed that far from being self-luminous, the animal had also been on a collecting expedition and his hearty meal of fire-flies was shining through the belly with considerable intensity. Although the toad engulfed his fire-flies in toto, it is doubtful if a spider could swallow more than minute fragments.

Infection of insects with luminous bacteria will finally spread to all parts of an animal, but the early stages might involve only the abdomen. The spider observed by Brown could have been infected with luminous bacteria. However, a fact definitely in favor of a true luminous organ is the statement of Bompas that the spider from Assam "was in the middle of a bush, and, when approached or shaken, glowed more brightly" and "The spider is truly phosphorescent and switches on its light when frightened." On the other hand, luminous bacteria glow continuously, the luminescence being quite independent of stimulation. It is possible that some spiders are self-luminous but rather surprising that so few instances of spider luminescence have been recorded.

Pycnogonida

While dredging on the east coast of the Andaman islands, Alcock (1902) obtained from 922 fathoms a "gigantic sea spider (*Colossendeis gigas*) the span of whose lanky legs was nearly 20 inches, the creature as it lay on its back, shone like a star, all its legs being lit along their ventral surface with a strange greenish blue radiance." Alcock stated that *Colossendeis* is blind and feeds like an earthworm on mud so that the use of luminescence is quite a problem unless it serves to scare enemies. No more recent instances of luminous sea spiders have been reported and nothing is known of the structure of the luminous regions.

MYRIAPODA

Among the four orders of myriapods the Pauropoda, Diplopoda, Chilopoda, and Symphyla, the chilopods and one species of diplopod exhibit unquestioned luminosity. The luminescence of other diplopods, reported by a number of observers, is presumably bacterial in origin. The position of luminous groups (in italics) in a classification of Myriapoda by C. G. Attems is as follows:

Pauropoda (3 families)

Diplopoda (millipedes)

 Pselaphognatha

 Polyxenoidea (Polyxenidae)

Chilognatha

Limacomorpha (Glomeridesmidae)

Oniscomorpha (4 families)

?*Polydesmoidea* (16 families, including ?*Fontariidae* = *Xystodesmidae*)

Nematophora (28 families)

Juliformia (15 families, including *Spirobolidae* and ?*Trigoniulidae*)

Colobognatha (5 families)

Chilopoda (centipedes)

Geophilomorpha (10 families)

Scolopendromorpha (2 families)

Lithiobiomorpha (3 families)

Scutigeromorpha (Scutigeridae)

Symphyla (3 families)*Diplopoda*

Records of luminous diplopods or millipedes are relatively few and difficult to interpret. The old word *Julus*, used by Mouffet in 1634 and 1658 and "millipedes" by Réaumur (1723) probably referred to a centipede. An early observation of what might be a luminous diplopod was made by Bruner (1890), who noticed near Omaha, Nebraska, in the summer of 1877 "a double series of small beads of fire crawling about among the dead grass." He caught three or four and kept them at his home in Massachusetts where some laid eggs, 1.75 to 2 mm in diameter. "They were a many jointed affair with two pairs of legs to each joint, $1\frac{1}{2}$ – $1\frac{3}{4}$ " long, yellowish brown and had the edges of each segment margined with a narrow yellow line above. There were also two round yellow marks upon each segment dorsally, one near each lateral edge. These latter were about 1 mm in diam., and were the source of the phosphorescence when the animal was placed in the dark. The light that was emitted was whitish and, if it is remembered correctly, more marked or intense at one time than another." Professor Riley said they were *Phengodes* but Bruner maintained that he knew the difference between a "thousand legged" and a "worm."

Later Kenyon (1893), in his preliminary list of the myriapods of Nebraska quoted the statement of Bruner and added: "It would seem at first that the phosphorescence was due to the secretion of the repugnatorial pores, but Mr. Bruner informs me that phosphorescence was communicated to the hands upon handling the myriapods and that the bright spot was inside the edge of the carina." Kenyon was unaware of any luminous diplopod having been previously mentioned and suggested the name *Fontaria luminosa* of the *Xystodesmidae*. Cook (1900) cited the opinions of both Bruner and Kenyon in his study of repugnatorial secretions of diplopods and examined the specimens of Kenyon, but felt that "the generic affinity is rather remote."

Molisch (1904, p. 47), in 1902 found in his greenhouse an unidentified

lief luminous myriapod, a diplopod of the order Chilognatha. Later he searched for more specimens and caught two, but they were not luminous. It seems fairly certain that the diplopod of Molisch was infected by luminous bacteria.

Some cases of diplopod luminescence are known definitely to be due to luminous bacteria. Haneda (1939) mentioned a diplopod from Palau Islands, whose light lasted for a day. He has informed me that he cultured luminous bacteria from this millipede which is also common in the Caroline and Marshall islands, and whose name is *Trigoniulus rugosus*, of the *Trigoniulidae*.

Self-luminous millipedes have been found by Haneda in Truk Island in 1939. They were sent to Takakuwa (1941) who described them as *Spirobolellus phosphoreus*, a new species very similar to *S. chrysogrammus*, belonging to the *Spirobolidae*. Haneda reported that the whole body was weakly bluish white luminous except the head and legs. When irritated, the light became bright, but no luminous material was ejected, as in luminous centipedes.

Finally the author received a letter in June, 1949, from Dr. Demorest Davenport of Santa Barbara College, California, telling of a brightly luminous diplopod, collected by students in the Sierras at middle altitude. "The antennae, legs, and edges of the dorsal sclerites glow with a diffiuse light." Again a luminous bacterial infection is suspected but not proved. The specimens were identified by Dr. H. F. Loomis of the U.S. Department of Agriculture as a new species, *Luminodesmus sequoiae*, belonging to the family *Xystodesmidae*.

Chilopoda

Probably the earliest record of luminous chilopods is that of Oviedo, who found them on the island of St. Domingo in 1520. Since that time luminous myriapods have been mentioned by such early naturalists as Mouffet, Garman, Ray, Willoughby, Réaumur, Fougereux de Bondaroy, Linné, DeGeer, and Fabricius. In the nineteenth century luminous centipedes were known to Shaw (1806), Macartney (1810), Audouin (1840), Newport (1845), Bedel and Simon (1875), Fanzago (1879, 81), Brodhurst (1880), MacLeod (1880), and the various writers on luminous animals. Richard (1885) has given the history of these early discoveries.

Important information regarding light production begins with Pascerini (1882) on *Geophilus gabrielis*. Studies have been continued by Dubois (1886, 87, 93) on *Scolioplanes crassipes* and *Orya barbarica*; Mace (1886) on *Geophilus simplex*; Gazagnaire on *Orya barbarica* (1888) and other forms (1890); Ludwig (1901) on *Scolioplanes cras-*

sipes; Barde-Birks (1920) on *Geophilus carpophagus*; Arndt (1924) and Koch (1927) on *Scolioplanes crassipes*. The most complete accounts are by Brade-Birks and Koch.

Additional records of luminous centipedes in Europe are due to Huet (1886), Haase (1889), Sinclair (1895), Thomas (1895, 1902), Brockhausen (1903), Haupt (1903), Verhoeff (1908), Roberts (1916), and Ridley (1936). Most observers remark on the brightness of the light, and Roberts has related that when troops marched across damp grassland to the trenches in northern France in 1916, the centipedes were so numerous and so bright that officers thought the men had disobeyed the no smoking order which had been passed along the column. Their appearance is shown in Fig. 119.

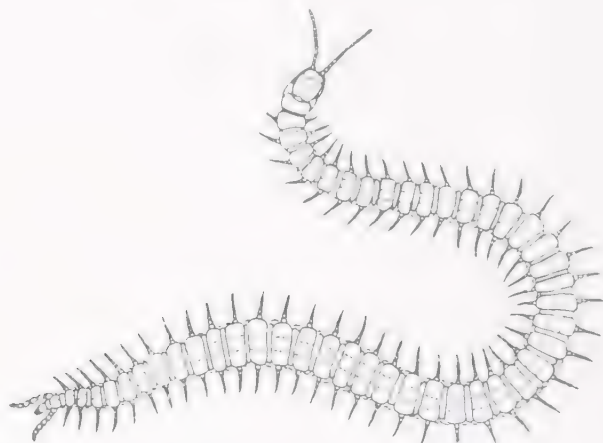


FIG. 119. *Geophilus carpophagus*. After Brade-Birks.

Luminous centipedes have also been found in Algeria (Gazagnaire, 1888; Dubois, 1893) and in Java, where the author has seen them. They were reported as among the forms reappearing on Krakatoa (Jacobson, 1908; Dammermann, 1923) after the volcanic explosion. Haneda (1939) has observed *Orphaneus brevilabiatus*, which is widely distributed in Micronesia and the East Indies.

It is difficult to identify certain species from early descriptions, but Verhoeff (1908) has listed *Scolioplanes crassipes*, *Geophilus electricus*, *G. carpophagus*, and *G. longicornis*. *Orphnaeus brevilabiatus*, *Stigmatogaster subterraneum*, and *Orya barbarica* as probably luminous. The luminescence of some species of *Geophilus* is perhaps doubtful as Koch never found a luminous example, among many observed animals. It is common for one observer to report an animal luminous and another to deny it.

Another chilopod, *Otostigmus aculeatus*, of the Scolopendridae, is

a pest in Tonkin during the hot season. Patton (1931, part II, p. 691), in his book on Insects, Ticks, Mites and Venemous Animals has written that, when touched, *Otostigmus* emits "a phosphorescent vesicant fluid, said to cause painful blisters of the skin lasting fourteen days."

The luminous genera and families (in italics), confined to the Geophilomorpha and Scolopendromorpha, are as follows in the classification of C. G. Attems:

Geophilomorpha

Himantariidae (10 genera, including *Himantarium* and *Stigmatogaster*)

Schendylidae (18 genera)

Oryidae (11 genera, including *Orya* and *Orphaneus*)

Mecistocephalidae (7 genera)

Geophilidae (61 genera, including *Geophilus* and *Scolioplanes*)

Soniphilidae (*Soniphilus*, *Poaphilus*)

Neogeophilidae (*Neogeophilus*, *Evallogeophilus*)

Azygethidae (*Azygethus*)

Gonibregmatidae (6 genera)

Sogonidae (*Garrina*, *Sogona*, *Timpina*)

Scolopendromorpha

Scolopendridae (23 genera, including *Otostigmus* or *Otostigma*)

Cryptopidae (12 genera)

Luminescence and Breeding Season. The question of luminescence in a particular species of myriapod is complicated not only by improper identification but also by the possibility that these animals may be luminous only at certain times of the year. In Europe, Gazagnaire (1890) has noted that most records of luminous animals are dated between the middle of September and the middle of November, a time that corresponds to the breeding season. Both sexes are known to be luminous.

According to Fabre there is no coitus among geophilids but spermatophores are deposited in passages in the soil and the female must collect them to fertilize herself. Since geophilids have no eyes, it is a priori difficult to understand why luminescence should appear only at the breeding season when the purpose of the light might be expected to be the attraction of male and female.

In fact, later observations have made the seasonal appearance of luminescence rather doubtful. Brade-Birks (1920) has reported that luminous specimens of *Geophilus carpophagus* were taken in Kent, England, in December, January, February, and April and that captive specimens were luminous from April to September. Koch (1927) also has come to the conclusion that myriapods can luminesce the year round. The reason they are recorded as luminous during the sexual season is because they come out from the earth at that time and are

most likely to be caught and the luminescence observed. That competent observers have often found a known luminous species to be non-luminous can be explained by the fact that myriapods light only on stimulation and, once having exhausted their secretion, at least three to four weeks must elapse before the luminous material is reformed (Koch, 1920). If a centipede happened to be captured during this period, it would naturally be classed as a non-luminous form.

Use of the Light. Absence of eyes in geophilids makes it unlikely that the luminescence is for recognition or a sex attraction. It is probably used for protection. Thomas (1902) has observed a luminous centipede scattering its secretion over red ants which had attacked it, and Brade-Birks also noticed that in a vial with ants the secretion was immediately formed. Ridley (1936) described an attack by a beetle, *Harpalus ruficornis*, which had seized the centipede and shook it as a terrier does a rat. "The beetle's mouth, face and legs were covered with patches of luminous matter which seemed to annoy it." Ridley also quoted from a letter published in Kirby and Spence's *Entomology*¹ as follows: "Mr. Sheppard once noticed a *Carabus* running round the last mentioned insect [*Geophilus electricus*] when shining, as if wishing but afraid to attack it." Koch has also described the behavior of a *Scolioplanes crassipes* in a glass dish with a beetle. When the beetle came near the myriapod and touched it with its antennae, the myriapod lighted momentarily and then turned the underside of some segments of the body against the beetle and covered it with luminous slime. A number of observers have noted the peculiar smell of the secretion and also that it is acid. Hence, the combination of a poisonous repellant with luminescence would be an excellent protective device.

Morphology and Histology. Although Ludwig (1901) had suggested that the light of *Scolioplanes crassipes* might be due to luminous fungi and Verhoeff (1908) and Buchner (1921) were inclined to agree, there is no doubt of the true luminescence of these animals. A secretion is formed which sticks to the fingers and to objects over which the centipede crawls. Dubois (1886) at first believed that the luminescent secretion came from epithelial cells of the alimentary canal but the work of Mace (1886), Gazagnaire (1888), Dubois (1895) himself, Brade-Birks (1920), and Koch (1927) has indicated that in all species hypodermal gland cells manufacture the luminous material, which is secreted to the exterior through pores (Fig. 121). Both Brade-Birks and Koch have described the distribution of these gland cells, which can be seen as whitish patterns under the integument. These patterns correspond to the six regions of luminescence forming a U on each seg-

¹ Peoples edition. London, 1867, p. 409.

ment, observed by eye or recorded on a photographic plate by momentary exposure to the luminescent animal. Such a "photogram" is shown in Fig. 120. Microscopic study has revealed six definite pore fields, also forming a U, through which the excretion finds its way to the exterior. In *Scolioplanes crassipes*, gland cells occur on the ventral side of all segments from the second to the fourth from last.

Histological study by Brade-Birks and Koch has revealed the existence of two kinds of gland cells. First there are the white gland cells seen through the integument that presumably contain "protoluciferin" and that are full of large eosinophil granules, and second, intimately mixed with them, non-staining fine granular mucous cells. The first type vary greatly in appearance, depending on the stage of regeneration of the secretion. Brade-Birks noted the almost complete disap-

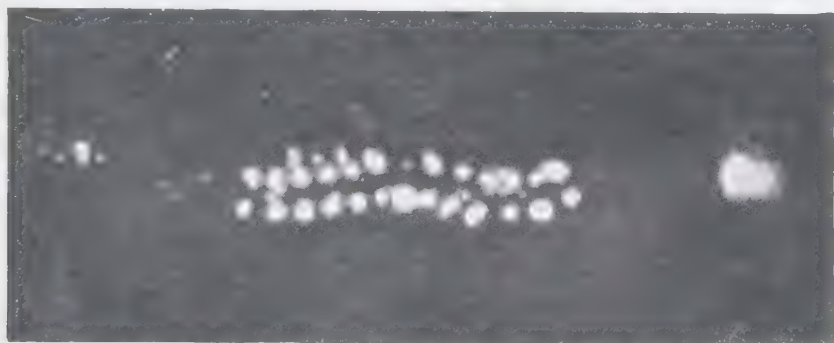


FIG. 120. The effect of the luminous secretion from *Geophilus carpophagus* on a photographic plate, showing local areas of formation. After Brade-Birks.

pearance of the white glands after a particularly extensive display of luminescence. Both types of cells open to the under surface through separate pores. They measure about $140\ \mu$ long and 45 to $70\ \mu$ broad and are shown in Fig. 121.

The secretory stages in *Scolioplanes crassipes* have been minutely studied by Koch, who has compared them with similar types of gland cells found in a non-luminous centipede, *Geophilus linearis*. In this form the gland cells are also whitish and visible through the integument, and on stimulation the white material is secreted through pore fields. Moreover, two types of cells are found in histological preparations of *G. linearis*, a fine granular type, staining weakly in eosin, and a more watery clear protoplasmic mucous type. Since the mucous cells of luminous and non-luminous centipedes are very similar and the eosinophil cells differ somewhat, Koch believed the latter to be the cells forming the luminous secretion.

The milky appearance of the white glands indicates that they are

full of globules. These bodies can be made out in stained sections and are clearly visible in the fresh secretion under the microscope. Dubois (1886) was the first to study them carefully, giving them the name vacuolides, since they contain a little vacuole in the center, and pointing out that they were not fat but protein in nature. Although characteristic of the luminous cells of many organisms, vacuolides are particularly prominent in the gland cells and secretion of *Orya barbarica*. They are called "gouttelettes" by Dubois (1893). He described the formation of crystals from the central spot or vacuole and at one time believed that the light of myriapods was a crystalloluminescence.

Gazagnaire (1888) also noticed the vacuolides of *Orya barbarica* and Koch (1927) has carefully described the crystal formation in the

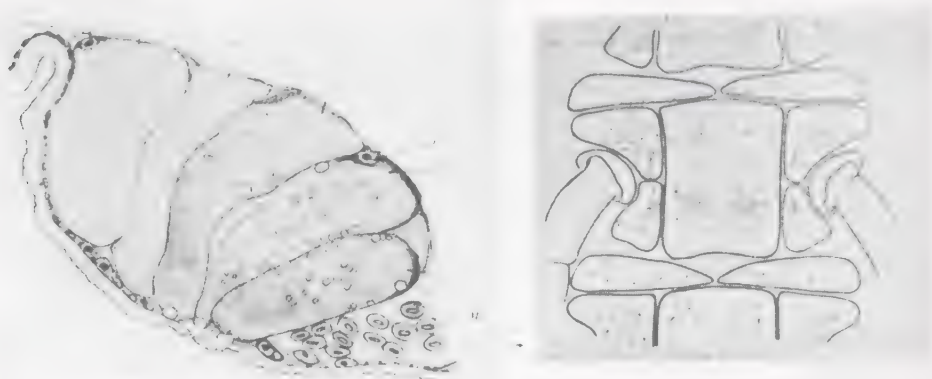


FIG. 121. Right, distribution of gland pore openings on the under surface of a segment of *Scoliplanes crassipes*. Left, a section of the gland cells. After Koch.

central vacuole of *Scoliplanes crassipes*. The crystal is first recognizable a few minutes after secretion as a birefringent structure in the vacuole, and in fifteen minutes the outline of the crystal with its "black cross" between crossed Nicols is clearly visible. Since crystal formation is also found in the secretion of *Himantarium gabrielis* and *Geophilus insculptus*, both non-luminous forms, Koch believes the crystals have no connection with the luminescent process. Brade-Birks (1920) also noted crystals in the secretion of both luminous and non-luminous myriapods and found that a *Geophilus carpohagus*, when stimulated electrically under water, produced a bright luminous secretion.

Physiology and Biochemistry. The usual method of stimulation is mechanical, probably a reflex involving the central nervous system, since Thomas (1895) has described a wave of darkness sweeping over the illuminated animal from tail to head. Apparently nerves do not supply the luminous gland cells but in non-luminous geophilids a

muscle layer over the glands acts on contraction to force secretion out of the pores, and this musculature is undoubtedly controlled by nerves and is active in luminous species also. Koch observed, as did Dubois, that a weak stimulation resulted in luminescence without the expulsion of a luminous secretion, leading Koch to believe that luminescence is initially intracellular, but strong stimulation by mechanical, electrical, thermal, or chemical means evokes the luminous secretion.

On the other hand Brade-Birks (1920) never observed that intracellular luminescence might occur in *Geophilus carpophagus* but rather that on electrical stimulation a few seconds elapse before the luminescent secretion appears. It is very bright at first, but quickly falls to a lower intensity, and the light then slowly disappears over a period of thirty seconds. In other forms the light of the secretion lasts a longer time. With the microscope, Brade-Birks was able to observe the contents of the "white gland" cells squirted through a narrow neck, like that of a bottle, and luminescence to appear a fraction of second later.

Brade-Birks described the viscous secretion as practically colorless with a fruity smell and strongly acid in reaction. Gazagnaire (1890) referred to the secretion of *Orya barbarica* as yellowish with a blue-green luminescence, while Koch (1927) described the milky secretion of *Scolioplanes crassipes* as weakly fluorescent, bluish in reflected light and weak yellow in transmitted light. Koch found a few bacteria in the secretion, but was unable to culture any luminous forms and was satisfied that the light was not bacterial in origin. The luminescence is an intense green, but spectral characteristics have not been worked out for any of the luminous myriapods.

The light of geophilids is undoubtedly a chemiluminescence, not a crystalloluminescence, and studies on the nature of the luminous material should be undertaken. Brade-Birks found that the secretion would luminesce brightly under water which had been previously boiled to remove oxygen and then cooled, but this observation may merely mean that enough dissolved oxygen remained in the water to maintain the luminescence. Dubois had previously shown that oxygen is necessary for luminescence and also that the secretion may be dried and will again luminesce on moistening.

The author (1931) was unable to demonstrate the luciferin-luciferase reaction with one specimen of a geophilid at Buitenzorg, Java, but additional tests should be made. In fact the chemistry of myriapod luminescence is quite untouched and offers a very promising field of investigation.

CHAPTER XII

Insecta Except Coleoptera

CLASSIFICATION

Among insects luminescence may be due to infection with luminous bacteria or to the possession of true luminous organs. Definite cases of infection are to be found among the midges and caterpillars, for luminous bacteria have actually been isolated and grown from these forms. The origin of luminescence of an ant, of may-flies and of a mole-cricket is less certain, but luminous bacterial infection seems highly probable. Although the original descriptions make it impossible to speak with certainty about these cases, the work of Pfeiffer and Stammer (1930) has demonstrated the ease with which luminous insects can be created by injection of luminous bacteria. The records of luminous termite hills are probably to be explained by luminous animals living on them.

True instances of self-luminescence are to be found in the Collembola (spring-tails), the Hemiptera (lantern-flies), the Coleoptera (fire-flies, glow-worms, and elaterids) and the Diptera (fungus-gnat larvae). The reported luminescence of secretion from glands of the prothorax of the tiger-moth, *Arctia caja*, has yet to be substantiated. The apparent light from the eyes of moths flying about a flame is due to reflection, as Panzeri (1872) has pointed out, and it is very probable that a similar explanation can be applied to certain white nocturnal wasps, described as luminous by Schultz and Stern. Alleged luminosity has also been attributed to a few beetles, not belonging to the elaterids or the lampyrid group, and to an orthopteran, of the grasshopper family (Brunner, 1908).

When the cases of true self-luminosity among insect orders are sifted from the false, it is apparent that relatively few of the huge variety of forms have evolved a light-producing mechanism. Nevertheless the insects which do light, common fire-flies, for example, are very abundant and of worldwide distribution. In the following classifica-

tion, based on Imms,¹ only orders with self-luminous species are italicized.

Insecta

Diplura (3 families) Campodea
 Thysanura (5 families) Silver-fish or bristle-tails
Collembola (4 families) Spring-tails
 Protura (2 families) Acerentomon and Eosentomon
 Orthoptera (27 families) Cockroaches, grasshoppers, crickets, mantids
 Dermaptera (3 families) Earwigs
 Isoptera (2 families) Termites
 Plecoptera (Perlidae) Stone-flies
 Embioptera (Embiidae)
 Psocoptera or Corrodentia (3 families) Book-lice
 Odonata (5 families) Dragon-flies
Hemiptera (55 families) True bugs
 Ephemeroptera (Ephemeridae) May-flies
 Anopleura or Siphunculata (5 families) Biting and Sucking lice
 Thysanoptera (2 families) Thrips
 Neuroptera (19 families) Lace-wing flies, Ant lions, Alder-flies
 Mecoptera (5 families) Scorpion-flies
 Trichoptera (15 families) Caddis-flies
 Lepidoptera (89 families) Moths, Butterflies
Coleoptera (109 families) Beetles
 Hymenoptera (43 families) Bees, Wasps, Ants
Diptera (117 families) Flies
 Aphaniptera or Siphonaptera (5 families) Fleas
 Strepsiptera (3 families) Stylops

Practically every textbook of entomology contains a chapter on luminous insects, and all general works on bioluminescence treat them, particularly Mangold (1910) and Dahlgren (1916). Gadeau de Kerville published his *Les Insectes Phosphorescents* in 1881 and 1887, and Maluf reviewed briefly arthropod light production in 1937-38, but no recent work has collected the accumulated knowledge of luminescence in this fascinating class as a whole. The monograph of Buck (1948) deals only with fire-flies and glow-worms.

COLLEMBOLA

The first published observation of luminescence among the spring-tails appears to be that of Allman (1851), who noticed the light of *Amurophorus fimetarius* (*Leptura fimetaria* or *Aphorura fimentaria*) on a hill near Dublin. Dubois (1886) has also recorded them from Heidelberg, a form like *Lipura armata*² with a bluish light, and Molisch

¹ A. D. Imms, *Outlines of Entomology*, New York, 1942.

² According to Ludwig (1904) this was probably *Aphorura armata* = *Onychiurus armatus*. The early nomenclature is confusing.

(1904) a species from Prague called *Neanura* (*Achorutes*) *muscorum*. Barber (1913) observed two different species from the Virginia shore of the Potomac River to be luminous, a relatively large one, *Anurida* sp. which luminesced greenish yellow continuously from the whole body, and a smaller species 1 mm long, *Neanura quadrioculata*, that flashed. The continuous luminescence of *Anurida* agrees with the description of *Anurophorus* by Allman and of *Lipura* by Dubois, while the flashes of *Neanura quadrioculata* agree with Molisch's *N. muscorum*. Both Barber and Molisch noted that light of the forms which luminesce on stimulation lasts for some seconds and then goes out and that the animals will luminesce again after a period of rest, but they fatigue readily. Handschin (1921, 26) has investigated six species of

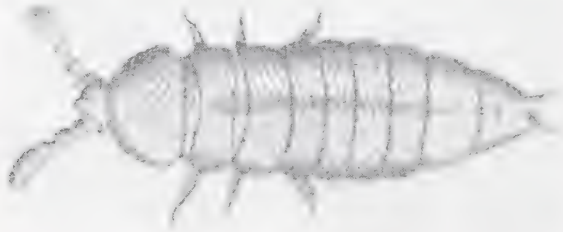


FIG. 122. The collembolid insect, *Lipura*. After Henneguy.

Collembola in Europe but found only one, *Achorutes muscorum*, to luminesce. He was unable to observe light in *Onychiurus armatus*, which Heidt (1936) later described as luminescing brilliantly. Both Heidt and Stammer (1935) have also investigated *Achorutes muscorum*. One of these forms is shown in Fig. 122.

The position of these genera (in italics) in a classification of the Collembola by A. Handlirsch is as follows.

Collembola

Poduridae

Podurinae (30 genera and 300 species, including *Podura*, *Xenylla*, *Hypogastura*, *Friesea*, *Pseudachorutes*, *Odontella*, *Anurida*, *Neanura* = *Achorutes*, *Onychiurus* = *Lipura* or *Aphorura*, *Tullbergia*, and others)

Entomobryinae (50 genera and 560 species including *Anurophorus*, *Folsomia*, *Proisotoma*, *Isotoma*, *Isotomurus*, *Oncopodura*, *Entomobrya*, *Sinella*, *Sira*, and others)

Actaletidae (*Actaletes*)

Smithuridae

Neelinae (*Neelus* and *Megalothorax*)

Smithurinae (13 genera and 200 species including *Smithurinus*, *Bourletiella*, *Dicyrtoma*, *Corynephoria*, and others)

The origin of the light has been discussed by such early observers as Dubois, Molisch, and Ludwig. Ludwig believed, since collembolids live in damp wood mold which usually contains luminous mycelia of various fungi, that the light came from fungi which have been eaten by the collembolids, and Molisch was inclined to favor this view. From what is known of bacterial and fungal luminescence this theory might apply to those forms with continuous luminescence but seems unlikely in the species which light only on stimulation. Dubois took the opposite point of view. He found *Lipura armata* to behave like most luminous animals. The body fluid was acid and contained crystals like those of *Pyrophorus* and *Lampyrids*.

Although Handschin was a specialist on Collembola and prepared the article on the group for P. Schultz's *Biologie der Tiere Deutschlands*, he was unable to observe any light in *Onychiurus armatus*, *O. fimentarius*, *Kalaphorura burmeisteri*, *Anurida granularia*, or *Folsomia fimentaria*, despite the fact that they were tested in darkness and under different colored lights, at different temperatures and in an oxygen stream. In a second paper (1926), he confirmed the bluish luminescence of *Achorutes muscorum*, obtained in the mountains of the Engadin, Switzerland. The light was very bright, visible at one meter distance but after some days disappeared gradually, beginning at the mouth and proceeding backwards. The animals live in fungus-infected wood, and he found the digestive tract to be filled with luminous fungal mycelium whose light was visible through the skin, especially where pigment was lacking. Moreover, *Achorutes muscorum* from other localities was not luminescent. All these facts led Handschin to believe the luminescence was due to fungi.

In recent years Stammer and Heidt have carried out experiments which indicate the light of Collembola to be a true self-luminescence. Stammer investigated *Achorutes muscorum* in the forests near Breslau and near Malchin in Mecklenburg. They showed no trace of light until shaken in a container when a relatively bright and uniform light appeared from the entire body, lasted for five to ten seconds, and then went out. Fatigue set in easily. Stammer found no secretion and by analogy with the gnat, *Ceroplatus*, he thought the light a true intracellular self-luminescence coming from the fat body. The eggs and young stages do not luminesce.

Heidt has made a very careful study of the luminescence of *Onychiurus armatus* and a few observations on *Achorutes muscorum*. The former was found in forests the year round near Giessen, Germany, where decaying leaves served as a medium for the growth of luminous fungi. However, the luminescence of the animal, which

is a continuous blue green light becoming brighter when the animals move about, remained undiminished after the *Onychiurus* had been kept on sterile agar for eight months. This observation indicates that the light is not the result of feeding on luminous fungi and not due to infection with parasitic luminous bacteria which invade and kill the hosts relatively quickly. The light was also much brighter than fungal light and was not restricted to the alimentary canal but shone throughout the body.

In CO_2 the resting light became much brighter and then disappeared; in chloroform and ether the light also slowly disappeared. Both effects were reversible. When placed in a vacuum, the light disappeared but returned with greatly increased brilliance when air was readmitted. The light was no brighter in pure oxygen than in air but the addition of H_2O_2 greatly increased the light intensity, an effect quite different from that of H_2O_2 on luminous bacteria.

On cutting the animal transversely in two, the rear portion luminesced more brightly and for a longer time. By squeezing the animals with forceps, it could be seen that drops of a luminous secretion appeared from the dorsal side. A few rod-shaped bacteria were found in this secretion, but not nearly enough to account for its bright luminescence, and all attempts to grow luminous bacteria from the secretion were negative. It seems clear that neither parasitic nor symbiotic bacteria are present in *Onychiurus armatus* Tbg.

Together with *Onychiurus armatus*, Heidt found two other species, *O. furcifer* C. B. and *O. fimentarium* L., living in the same habitat of luminescent mold. These forms were non-luminescent and could not be made luminescent when smeared with the luminescent material of *O. armatus*. Heidt also observed a few specimens of *Achorutes muscorum* which, as both Molisch (1904) and Stammer (1935) have noted, only luminesced on strong stimulation and no secretion of luminous drops could be detected in the three animals under observation.

There seems to be only the evidence of Handschin that the light of *Collembola* is due to luminous fungi although it has been noted that one species will luminesce and a closely allied species not. In addition, one observer has been unable to confirm the observation of another regarding light production in the same species. These conflicting results suggest the possibility that luminescence in *Collembola* only appears at certain times of year. The subject still awaits investigation as does also the possible use of the light to these tiny insects. The histology of the luminous cells has not been studied, nothing is known of the chemistry of luminescence, and no tests have been made for the luciferin-luciferase reaction or the possible part played by adenosine triphosphate.

ORTHOPTERA

In Kirby and Spence's (1817) *Introduction to Entomology* there is an account of a luminous mole-cricket caught by a farmer in 1780 and brought to Dr. Sutton at Iskelton, Cambridgeshire, England. No further mention of luminescence in this animal occurs until 1891 when F. Ludwig of Greiz reported a similar find by two of his pupils. The animal emitted a bright greenish white light from an asymmetrical spot on the right side of the body behind the head. The characteristics of the light led Ludwig to believe that infection with luminous bacteria had occurred, similar to the cases reported for midges. Even with the meager evidence at hand there is no reason to doubt this conclusion, for Molisch (1904, p. 78) kept many mole-crickets in his laboratory in the summer of 1901 without observing any evidence of luminescence.

The only other possible case of orthopteran luminescence is based on examination of dead specimens. In a footnote, Brunner (1908) cited a grasshopper (*Optoceras margaritatus*) of British Guiana as possibly nocturnal and luminescent from "pearly granules or follicles, which adorn the metapleura or hind femora," but he added that this is "only a surmise on the part of the present writer." The follicles probably reflect light.

ISOPTERA

There are several references to luminous termite nests in the literature. Castelnau (*Expédition dans les parties centrales de l'Amerique du Sud, Histoire du Voyage*, Paris, 1850, Vol. II, p. 103) says "in the vicinity of the Agoa Limpa estate near Goyaz, we noticed a luminous mass in the middle of the campo—a termite mound from which shone a great number of small points of light (petits foyers lumineux). This phenomenon is produced by the presence of an immense number of small phosphorescent larvae which withdrew into the galleries they had built when one tried to capture them."

Another instance is recorded by Dr. João Severiano de Fomeca (*Viagem ao redor do Brazil*, 1875-78, p. 353) "On the head waters of Rio Verde (state of Matto Grosso, Brazil) we saw one night a surprising sight. One of the white ant's nests seemed to be covered with little lights. . . . When the nest was struck with a stick the miniature lights went out as if by enchantment, but only to reappear again little by little, beginning where the blows had been weakest."³

In 1879, Smith, in his book, *Brazil, the Amazons and the Coast*, again refers (p. 139) to luminous termite nests, saying, "There are white ant hills along the sides—pale glows of phosphorescent light.

³ Quoted from Branner (1910).

like coals in the ashes" and in a footnote, "The phosphorescence is in the insects, and I believe it is peculiar to one or two forest species." Knab (1895, 1909) observed the same phenomenon during a walk through the forest near Santarem, Brazil, where a large termite hill seen through the foliage presented "a luminous area composed of innumerable points of phosphorescent light which appeared to shift and fuse into each other, thus forming more brilliant patches which were constantly resolving themselves and again appearing."

These descriptions mostly indicate that small animals on the nest are luminous, but there is no record of what they are and no certainty that they are luminous termites. Branner (1910) quoted Dr. Joaquim Lustosa, a Brazilian mining engineer, as saying that in the upper part of Matto Grosso abandoned white ant nests are often covered with fire-flies that live in small openings over the whole surface of the ant hill.

The best evidence is against the view that the light is emitted by termites, which are not accustomed to appear outside of their nests, and in favor of some adventitious insect living or collecting on the hills, perhaps a glow-worm or a fire-fly. The author ventures another suggestion, that the light may come from dipterous larvae, whose habitat and habits would agree quite well with the description of the lights given by the early observers.

EPHEMEROPTERA

In 1873 Hagan recorded a luminous may-fly, *Caenis dimidiata*, caught at Neuhausen not far from Pillau. Eaton (1880) referred to the above may-fly as emitting a pale blue light and in 1882 described another may-fly of the genus, *Teloganodes*, obtained by Mr. G. Lewis 1,500 feet high in the hills at Kitugalle, Ceylon. The whole abdomen of this form was luminous, although not very bright. It seems most likely that these insects were infected with luminous bacteria.

HEMIPTERA

Reported cases of luminosity among hemiptera are to be found in the suborder Homoptera, a group containing among others plant lice, scale insects, cicadas, lantern-flies, tree hoppers, spittle insects and leaf hoppers, as distinguished from "true bugs," none of which are luminous. One case has turned out to be a definite hoax, one of the few in luminous literature, a leaf-hopper, alleged to be *Tettigonia quadrivittata*. It was perpetrated by Rathvon (1870, p. 371) in the *American Entomologist*. Rathvon referred to a previous article (p. 335) on "An electrical insect" and said that he had seen "apparent electric sparks emitted from the end of the abdomen of a common species of *Tettigonia*

... noticed it on dark cloudy days. . . . These flashes or scintillations occurred about every 5 seconds and continued at those intervals for $\frac{1}{2}$ hr." With a magnifier Rathvon "found the tiny flash to proceed from an almost transparent member, which the insect quickly protruded from a caudal segment and as quickly withdrew." The editors of the *American Entomologist*, C. V. Riley and G. Vasey, said the animal sent them by Rathvon was *Diedrocephala coccinea* and expressed grave doubts of the veracity of the report.

The most famous case of hemipterous luminescence is to be found among the Fulgoridae, whose position in a classification of the Homoptera by M. Beier is as follows:

Hemiptera

Heteroptera

Homoptera

Cicadinea

Tettigometridae

Fulgoridae (13 subfamilies, including Fulgorinea with *Fulgora*, ?*Pyrops*, *Hotinus*, *Aphana*, *Euphria*, *Polydictya*, *Lystra*, *Phenax*, and many other genera)

Cercopidae (4 subfamilies)

Cicadidae (3 subfamilies)

Jassidae (6 subfamilies)

Aethalionidae

Membracidae (2 subfamilies)

Psyllinea

Psyllidae (5 subfamilies)

Aleurodinea

Aleurodidae

Aphidinea

Aphidae (4 subfamilies)

Coccinea

Coccidae (13 subfamilies)

Luminescence of the Fulgoridae has been a controversial matter for 250 years. Some thirty papers have contained evidence for or against light emission of the lantern-fly (*Fulgora lanternaria*), the fulgore or porte-lanterne of the French, found in tropical South America and not to be confused with a fire-fly. Other species (*Fulgora* or *Pyrops candeleria*) from tropical China and other genera from Africa have been called luminous and their light-emitting power has been discussed pro and con. About two-thirds of the reports on the "lantern-fly" deny the luminescence, and the rest affirm it.

The name lantern-fly is certainly suggestive, although it may have been applied from the shape of the lantern-like protuberance on the head rather than from observation of light emission. The insect is

shown in Fig. 123. The earliest illustration was made from a dead specimen from Peru in the museum of the Royal Society and appeared in Nathaniel Grew's *Museum Regalis Societatis* . . . published in London in 1681. The first individual actually to describe the luminescence from personal observation was probably the artist, Maria Sibille Meriam (1647-1717), who visited Surinam and studied new world insects between 1699 and 1701. Her book, *Metamorphosis Insectorum Surinamensium*, was published at Amsterdam in 1705.

A translation of her description reads: "The Indians brought a number of these insects which I put in a great wooden box. At night they made such a noise that I awoke with fear, not knowing what could



FIG. 123. *Fulgora lanternaria*. After Gadeau de Kerville.

have caused such a scuffle in the house, but soon realized that it was in the box. To my astonishment, on opening the box flames came out. Indeed the insects all lighted as if they were on fire and I was amazed by the splendor of these animals." Many early entomologists such as Rosel, Réaumur, and others accepted her statement which is certainly definite. Linné (1758) in the tenth edition of *Systema naturae* (p. 434) recognized five species, described under the name of *Cicada*, and grouped together as *Noctilucae*.

The more recent papers which deny luminescence of *Fulgora* either from personal observation or the statement of competent observers in the tropics are by Oliver in 1792, Hoffmannsegg (1807), Wied-Neuwied (1820), Spix and Marius (1824), Lefebure (1834), Hancock (1834), Westmail (1837), Westwood (1839), and White (1844) in China, Pillard (1864), Bates (1864), Becker (1848), Hagen (1853), Dolm (1868), Preyer (1880) in Borneo, Champion (1883), Branner (1885), Gounelle (1886), and Blair (1924). Burmeister and Lacordaire were

inclined to doubt the luminescence of fulgorids, while Kirby and Spence and Gadeau de Kerville favored the accounts of light production.

Donovan in 1798, in his *Epitome of the Natural History of the Insects of China* implied that *Fulgora candelaria* is luminous, but did not state that he actually saw it light, and Spinola (1839), in his monograph on the Fulgorelles, was likewise inclined to accept the luminescent qualities. Four nineteenth century writers, Stedman (1805), Wesmael (1838), Spence (1848), who quoted a Mr. W. H. Edwards at Para, Brazil, and Moufflet (1865), have stated unequivocally that *Fulgora lanternaria* is luminous and that the light comes from the head protuberance. Hagen (1865) later changed his opinion of non-luminosity, taking the stand that enough competent observers had seen the light to establish its reality and suggesting that perhaps it was a sexual characteristic and that only one sex was luminous.

The most recent observer is Mr. H. Heyde, an entomologist of Paramaribo, whose observations were reported in letters to Prof. O. Schneider-Orbeli of Zurich and to the author. Heyde obtained 3 males and 4 females in July, 1946, and placed them in a paper box. They flew about within, and when the box was opened Heyde saw that the head luminesced with a bright white light which soon switched off. When placed on the ground they did not light, and when males and females were separated they did not light. Only when placed together did they luminesce. The insects died in two days, but there appears to be no doubt that the luminescence is connected with mating. The light could not be due to a bacterial infection as the insects should then light when separated and in the daytime, which is not the case. The light came only late at night. Natives of this region often report seeing ghosts late at night, which might be these insects, and one night Heyde stationed himself at the reported place and did indeed see, about 11 p.m., a large light coming toward him which he caught with a net. It turned out to be a *Fulgora*. With the various confirmations of Meriam's statement there can be no doubt of the self-luminosity of *Fulgora*, especially as Heyde's observations supply the reason for the many negative reports. Unfortunately nothing is known of the histology, physiology, or biochemistry of the luminous cells.

That other fulgorids may be luminous is suggested by observations of Bell Marley (1913) in Durban, South Africa. One night he saw a light in the bushes and caught a *Rinortha guttata* which had lepidopterous larvae living on the under side of its wings. Although the title of his paper, "Some notes on a luminous South African fulgorid insect (*Rinortha guttata*), together with a description of its lepidopterous larva," would lead to the conclusion that this insect is luminous, there

is no statement of observation in a dark room or of the source or character of the light and subsequent attempts to find additional luminous specimens resulted in failure. Bell-Marley also mentioned that a friend had noticed a light at night when collecting in Zululand, which he attributed to a large fulgorid, *Pyrops*. The natives of Zululand called it "nkanyezi," a candle.

The luminescence of *Pyrops candelaria* of Asia has not yet been definitely settled. Newman (1864) has published a statement of Mr. James Smith that *Fulgora* (*Pyrops*) *candelaria* is luminous between May and August but not in winter, when it is only occasionally seen. "In summer it has a pale blue or green light at the end of the snout which may be considerably augmented by a gentle pressure of the insect; it is brightest in the female." It is common throughout all China, and called the "Star of Eye," "Eye of Confucius" or "Spark fly." Smith stated that the same insect is called in winter the flying elephant, perhaps in reference to its long proboscis and "when the insect is settled the light is more luminous than when it is flying, and when the male and female have mated it is wholly extinguished."

Kirkaldy (1901) quoted a Mr. Fletcher, who studied *Pyrops* in Southern China (Kaulung), where it is very common and known as the "Candle-fly," as stating categorically that it is not luminous. The name comes "from an old idea that the snout was luminous." Kershaw and Kirkaldy (1910) demonstrated the extension of the alimentary tract into the head of certain fulgorids, and Muir (1913) has suggested in a footnote: "The knowledge that this elongated head of *Pyrops* is filled with stomach may help to settle the much-controverted point as to the luminosity of this structure. It has been suggested that the light is due to bacteria, and as there is a luminous bacterium which lives in the stomach of silkworm larvae and makes the whole insect quite luminous, it is highly probable that bacteria in the stomach of *Pyrops* is responsible for the light seen on the head on rare occasions."

LEPIDOPTERA

Luminous Caterpillars

Records of luminous caterpillars go back to the observations of Gimmerthal (1829) of Riga, who found brightly phosphorescent larvae of *Noctua* (*Polia* or *Agrotis*) *occulta*. They remained luminous for eight days in captivity. Boisduval (1832) made a similar observation for caterpillars of *Mamestra oleracea*, and Holyroyde (1916) observed a luminous larva, probably of a noctuid moth, near Brighton, England.

Rye (1878) referred to both the above observations and then introduced a new type of record by quoting a description from the book by T. P. Bigg-Wither, *Pioneering in South Brazil*, published in 1878, of a luminous caterpillar with stinging hairs and light shining from each segment so that it looked like a railroad train.⁴ These animals were numerous in October and November near the Tropic of Capricorn, lining the borders of camps and lighting up the surroundings. Such a display has not been reported by Bates or other travelers in the region: the origin of the luminescence remains unknown.

However, there can be no doubt that occasionally caterpillars become infected with luminous bacteria and that Gimmerthal and Boisdual observed such specimens. The subject has been recently investigated by Stammer (1930) and by Pfeiffer and Stammer (1930) in an extensive monograph. These authors found a brightly luminous caterpillar of *Mamestra oleracea* near Steinau on the Oder and inoculated non-luminous caterpillars with the luminous blood. This fluid was swarming with a bacterium called *Bacterium hemophosphoreum*, quite different from three other described luminous bacteria, pathogenic for invertebrates. *B. hemophosphoreum* grew well on artificial culture media and could be transferred to *Agrotis* and *Pieris* caterpillars, as well as many other insects, both larvae and adults. No infection was obtained with saw-fly larvae, with *Smerinthus populi*, or with *Lampyrus noctiluca*, the European glow-worm. The resistance to infection of the European glow-worm is of particular interest. Attempts to infect earthworms, Gammarus, crayfish, frogs, and fishes (*Phoxinus laevis* and *Leuciscus rutilus*) with *B. hemophosphoreum* also failed, so that the bacterium appears to be particularly virulent for insects. It was not possible, however, to infect insects by mouth, that is, during normal feeding; they only succumbed to injection. Many studies on immune reactions were made.

Adult Moths

Among adult lepidoptera, the eyes of moths attracted to a flame, particularly sphinx moths, are often said to be luminous. Panceri (1872) published a special paper on this phenomenon, pointing out that although Dessaignes thought the glow of eyes was due to insolation and Pallas to electricity, Prevost (1810) had demonstrated that a reflection of light was responsible in the case of such night moths as *Sphinx atropos*. Panceri himself had experimented with a *Catocala elocata* moth and found no luminescence in a totally dark room. The

⁴It is possible that the "railroad worm" *Phrixothrix* was observed, but the statement that the animals were numerous makes this interpretation unlikely.

apparent light from insect eyes is a striking reflection phenomenon but not a bioluminescence.

Luminescence of the antennae of a noctuid moth, *Asteroscopus sphinx*, is in another category. Schultz (1899) captured such a moth found flying at Seeren in der Neumark. Both antennae were luminous, one for almost its whole length and the other near the middle. On squeezing one antenna, luminous material came out on his fingers. The other antenna remained luminous for over two days, when the moth suddenly died. Schultz thought the antennae had come in contact with some luminous material, but the long duration of the light indicates a luminous bacterial infection, which may have involved other parts of the body without being visible through the pigmented chitinous wall.

Arctia Caja

A very remarkable case of luminescence has been recorded by Isaak (1916) in the Great Tiger moth, *Arctia caja*, whose imago possesses two glands on the dorsal surface of the prothorax, which, when the animal is stimulated, secretes a greenish luminescent fluid. The description given by Isaak (1916) is perfectly definite. Ordinarily the gland openings on the prothorax are covered by long hairs, but on strong stimulation the hairs are spread apart and a drop of bright yellow (in daylight) oily liquid appears. This luminous secretion never appeared spontaneously, but could be evoked by squeezing head and thorax and was found in both sexes. The luminescence lasted about ten seconds, and by then the secretion had been absorbed.

In discussing this report, Hykes (1917) pointed out that the secretion of *Arctia caja*, which has a definite smell, has been observed many times but that no one noticed the luminescence. The author, also, has talked and corresponded with a number of entomologists who were unaware of any luminescence of *Arctia caja*. The most careful investigation has been made by a writer signing himself A.U.E. (1918, 22), who examined over two hundred specimens of *Arctia caja*, which produced the tiny droplets of secretion in the dark, but with no evidence of luminescence. It is most probable that Isaak saw light reflected in the spherical globules of yellow secretion.

Nevertheless Hepp (1827) has repeated the statement that when *Arctia caja* is disturbed, a light organ is exposed from which exudes a drop of pungent luminous secretion. This secretion is also observed in *Parasemia plantaginis*, and from tubercles of the larvae of *Eudia pavonia* and *Zygaena ephialtes* var. *peucedani*.

DIPTERA

Among the true flies, three groups have been described as luminous. One type of luminescence is found among adult gnats or midges, Chironomidae, and is undoubtedly an infection by luminous bacteria that ultimately leads to the death of the midge. The other groups contain larvae which are self-luminous. Among them are the larvae of fungus gnats (Platyuridae), Ceroplatus, and Platyura, and the famed New Zealand glow-worm, Boletophila (Arachnocampa) of the Boletophilidae.

Among false luminescences, Osten-Sacken (1878) in a review of luminous diptera quoted Marquart as saying that the head of the remarkable fly, Thyreophora cynophila, which feeds on carrion, is luminous,⁵ but this observation has never been confirmed and is probably incorrect. Robineau-Desvoidy (1849) has denied it. The iridescent eyes of the fly may have been mistaken for luminosity.

Another false luminescence was reported by Westwood (1854). A tipulid fly, Helobia brevicollis, was found near Windsor, England, covered with adhering particles of luminous matter. The presumption was that the insect had been in contact with the secretion of a luminous centipede, as has been occasionally described.

The position of these families in a classification of the Diptera by F. Hendel is as follows. Families and genera containing self-luminous forms are in italics.

Diptera

Nematocera (35 families) gnats, midges, mosquitoes, etc.

Tipuloidea (3 families) crane flies

Phyrnoidea (Phrynidae or Rhyphidae or Anisopodidae)

Fungivoroidea (fungus gnats)

Mycetobiidae (Mycetobia, Paleoplatyura, Mesocria)

Lygistorrhinidae (Lygistorrhina)

Manotidae (Manota)

Sciophilidae (3 subfamilies, many genera)

Fungivoridae or Mycetophilidae (Exechia, Allodia, Rhymosia, Cordyla, Phronia, Fungivora = Mycetophila, Zygomyia, and others)

Zelmiridae or *Platyuridae* or *Ceroplastidae* (Asindulum, *Ceroplatus*, *Zelmira* = *Platyura*, and others)

Macroceridae (Macrocera)

Ditomyiidae (Ditomyia, Symmerus)

Diadocidiidae (Diadocidia)

Bolitophilidae (*Bolitophila*, *Arachnocampa*)

Lycoriidae or Sciariidae (4 subfamilies and many genera)

Itonidoidea or Cecidomyioidea (10 families) gall gnats

⁵ Marquart's words are "tête phosphorescente d'un rouge orange."

- Cuclicoidea (Dixidae, Corethridae or Cheobaridae, Culicidae) mosquitoes
- Tendipedoida or Chironomoidea (Chironomidae or Tendipedidae and Heleidae or Ceratopogonidae) gnats or midges
- Melusinoidea or Simuloidea (Melusinidae or Simuliidae) black flies
- Thaumaleoidea or Orphnephiloidea (Thaumaleidae or Orphnephilidae)
- Blepharoceroidea (3 families)
- Brachycera (82 families) flies

Midges

Observation that gnats may be luminous goes back to the eighteenth century when Carl Hablitzl wrote a letter from Astrabad (Persia) on July 7, 1782, describing the phenomenon. The following lines from the letter are quoted by Pallas.⁶ "Besides this luminous insect [Lampyrus], which is of very frequent occurrence on the shores of the Bay of Astrabad, I have likewise had the occasion to observe that in the dark a light also emanates from the gnats (*Culex pipiens*, L.). In fact I noticed this last autumn and in the spring of the present year, since these insects had established themselves in multitudes on board our ships." The letter itself was published by Hablitzl in 1789.

The next record is by Alenitzin (1875), who also saw luminous midges during his trip to the Sea of Aral, near the mouth of the river of Amu Daria. These insects were later identified as a species of *Chironomus* and there is little doubt that Hablitzl's insect was also a chironomid, rather than a *Culex*. Brischke had in 1860 observed a luminous *Chironomus* in the river Kaduane in Pomerania. This work is referred to by Osten-Sacken (1878).

Kusenoff (1890) reported them from Lake Issykkul. These luminous midges were later observed and carefully studied by Schmidt (1894) who learned that they were usually abundant in early June at Prshewalsk. Schmidt, who has recorded the history of the subject, came to the conclusion that the light was bacterial in origin, similar to the luminous bacterial infection of sand-fleas (Giard, 1889), for the following reasons: (1) The greenish light is continuous and shines from all parts of the body, even the legs and antennae. (2) The animals that luminesce are weak and obviously about to die. (3) Both male and female *Chironomus* are luminescent and also some other flies (*Corethra*) in the neighborhood, but the same species (*Chironomus intermedius*) is not luminous in other places. (4) No definite luminous organ could be found in the body. However, Schmidt was unable to grow luminous bacteria from the midges of Lake Issykkul.

Henneberg (1899) described four midges which were continuously greenish luminescent from all parts of the body and when squeezed

⁶ Quoted from Austen's translation of Schmidt (1894).

exuded a luminescent fluid. He also was unable to grow bacteria from this liquid, but believed that the light came from bacterial infection. This observation was made on the banks of the Elba near Magdeburg, but the midges were never seen again in this or other places.

Tarnani (1908) again observed luminous midges on the river Bug, near Nikolajev, and in 1911 Issatschenko found that the light of midges at Warwarowa was due to infection with *Bacterium chironomi*. This form could be grown on flesh-peptone-agar without salt, but gave a brighter light if 0.5 to 1.0% NaCl was added.

A more recent paper by Behning (1929) has recorded the light of *Chironomus behningi* from Lake Tschalkar on the steppes, 70 kilometers south of Uralsk, where many larvae were found in the brackish water. Among the numerous adults, Behning frequently found luminous midges resting on leaves or flying about. There can be little doubt of the regular occurrence of infected midges, and the experiments of Issatschenko (1911) have given definite proof that the light comes from bacterial infection.

Finally Lane (1951), in a note to the Entomological Society of Washington, has recorded mosquito pupae with luminous patches on the body emitting a purplish light. These patches remained on the pupal pelts after the adults had emerged, but the duration of the light was not determined nor is its cause known. The mosquito larvae (*Phonimomyia pallidoventer*) live in the water of epiphytic bromeliads of the forests of Brazil. Lane also found another species (*Culex inimitabilis*) with luminous spots on the first abdominal tergites. It is possible that the light came from the secretion of a myriapod, but further investigation will be necessary to determine its nature.

Ceroplatus

The more interesting luminous diptera of the family Platyruridae were probably first observed by Wahlberg (1849) in Sweden where the luminous larva of *Ceroplatus sesiodes* was found living on a web on the under surface of the mushroom, *Polyporus fomentarius*. The whole body is luminous in the larval and pupal stages although not in the adult. Osten-Sacken has given a description of the light which shone "through the cocoon as through a lantern." *Ceroplatus* differs from the New Zealand glow-worm, *Boletophila* (*Arachnocampa*) *luminosa*, and also from *Platyura* of the southern Appalachian Mountains in the large area which is luminescent. In both latter cases the light comes from definite organs in restricted regions of the body.

From the early descriptions it is not possible to tell whether or not

the *Ceroplatus* larvae were infected with luminous bacteria or had eaten luminous fungi, but later observations made the infection theory untenable. Pfeiffer and Stammer (1930) and Stammer (1933) have described luminous fungus eating *Ceroplatus testaceus* larvae living on webs under *Polyporus* (*Placodes*) *ungulatus* in the Heuscheuergebirge in May and June. When at rest the light was very weak and came from the whole body of larva, pupa, and also the adult for two days when the light disappeared. Stimulation of the animals by pressing or puncture increased the light intensity which was emitted by the hypodermal fat layer, although the fat around the alimentary canal was not luminous. In pupa and adult the same fat cells were luminous, and the disappearance of light in the adult was connected with disappearance of the fat body. The ovary or eggs or early larval stages were not luminous.

Stammer made a very extensive study of *Ceroplatus* and reported that neither in sections nor in smears nor in numerous attempts at culture could luminous bacteria be demonstrated. Related species of *Ceroplatus* larvae and the larvae of the fungus-eating genera, *Cerotelion*, *Zelmira*, and *Sciophila* were not luminous. Since luminous bacteria must be present in large numbers to emit a visible light and are easily detected, usually infect the whole body of the victim, and are likely to attack other species in the region as well, there is little doubt that *Ceroplatus* is self-luminous. The response to stimulation is additional, practically conclusive evidence against bacterial infection. The physiology and biochemistry of *Ceroplatus* are completely unknown.

Platyura

Closely related to *Ceroplatus* is an insectivorous lochetic dipterous larva of the genus *Platyura*. It has been described as luminous by Fulton (1939, 41) in several comprehensive papers. The animals have been observed around a spring near Glenville, North Carolina, at an altitude of 3,500 feet in the Appalachian Mountains. This form, *P. fultoni*, lives in wet, deeply shaded places near springs and streams and spins branching webs in the crevices between stones or in deep beds of moss. The larva feeds on small insects and is shown in Fig. 124. The light comes chiefly from the anterior end (5 anterior segments) occupying one fifth of the body length. In addition there is a small tail light on the posterior segment. Both lights have about the intensity of a lampyrid larva light, but are bluish rather than yellow in color. The light is continuous and independent of stimulation but disappears in daylight or artificial light, even though fairly weak.

According to Fulton there is a day-night rhythm of luminescence, the light disappearing in daytime and reappearing at night. This rhythm is also observed for a period of at least six days, if the larvae are kept in continual darkness. In nature the animals retreat into cavities

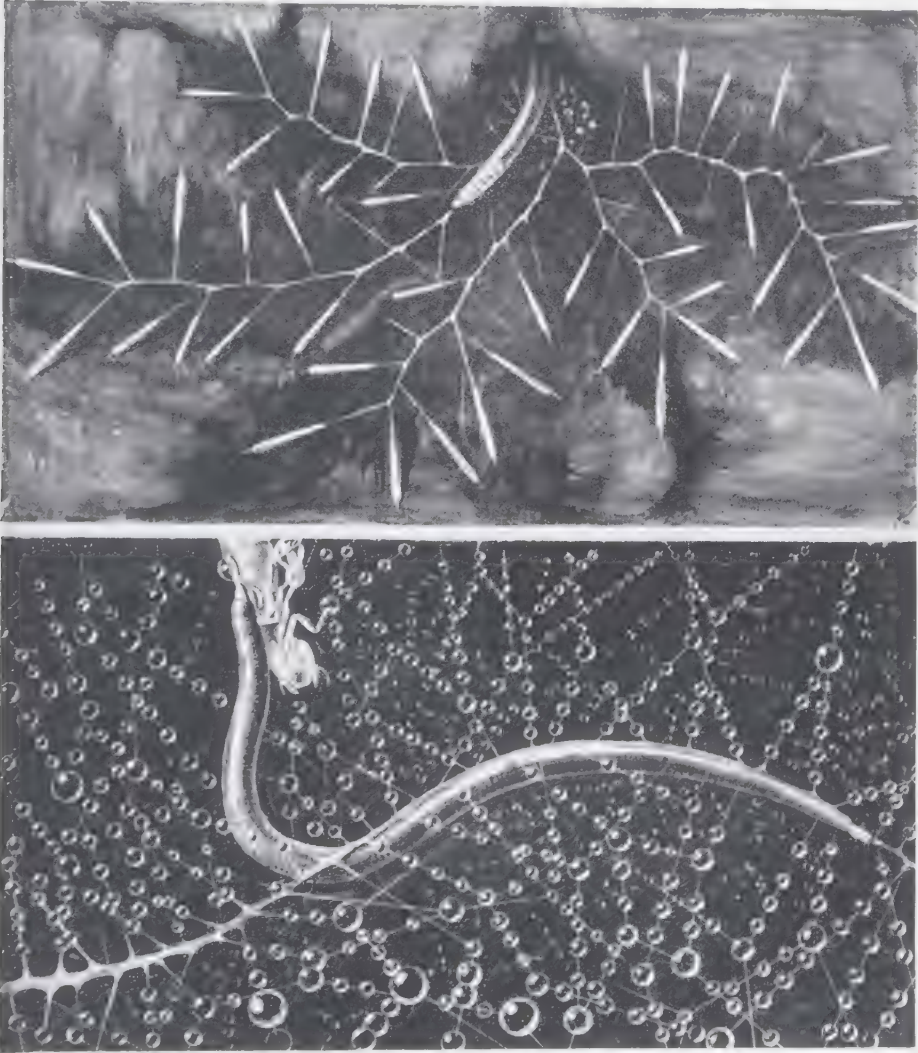


FIG. 124. *Platyura fultoni*, on its web (above) and an enlarged view of the larva with its prey (below). After Fulton.

in daytime but at night crawl out on the webs and expose their luminous bodies.

Morphology. The luminescence is connected with rows of small black structures, like loosely strung beads, which are visible through the unpigmented integument in the anterior and posterior luminous regions. The cuticle is brownish over the rest of the body. The

black bodies appear to be closed sacs well supplied with tracheal branches but having no other connections. If a larva is dissected under water and examined with a microscope in the dark, no light may be visible, but when one of the black structures is broken open, light does appear inside, and the luminous material spreads in the water. The luminescence seems to be brightest where the black pigment is most abundant.

On the other hand, when a luminous larva is examined with the microscope, the light does not appear to come directly from the black bodies, but the whole anterior and posterior regions of the body are illuminated. It is possible that a material is secreted from the black bodies into the body cavity. Further work will be necessary to establish this point.

Ecology. From the habits of these animals, which lie in wait for their prey in the webs they spin, then pounce on any small unwary insect moving in the web and cover it with a sticky slime, we may suppose the light to act as a lure. Although the larvae feed on any small insect that is caught in the web, the most abundant insects found in the natural habitat are Collembola.

The New Zealand Glow-worm

Although the New Zealand glow-worm must have been known for many years, the first scientific description was given by Meyrick (1886), who found larvae which he took to be those of a staphylinid beetle in a shaded creek near Auckland, New Zealand. They lighted with a "bright greenish white erect flame rising from the back of the neck." Hudson (1886, 87) quickly recognized that the insect was a fly larva, probably a tipulid and said the light came from a "large glutinous knob situated at the posterior extremity" of the larva which hangs in a glutinous web. Specimens were sent to the great dipterist, Osten-Sacken, who identified them as a mycetophilid, probably a *Sciophila*.

Hudson later (1891) reared the larva and his drawing is shown in Fig. 125. It was named *Bolitophila luminosa* by Skuse (1890, 91). Norris (1894) and Edwards (1924, 34) have added further to our knowledge, the latter changing the generic name to *Arachnocampa*. Hudson (1926) has summarized the early history and habits of the animal, which lives in New Zealand caves, the most famous being at Waitomo, 200 miles north of Wellington, where the lights of the larvae are a great tourist attraction. The larvae are also found in damp dark ravines such as one in the Wellington Botanical Gardens. The same insect or a related species is also found in caves in Tasmania at

Ida Bay (Ferguson, 1925) and at Bundannon in New South Wales and also in the Fiji Islands.

Visitors to the Waitomo caves have all been impressed with the splendor of the glow-worm display, and recent popular accounts have appeared by Blakeslee (1948) and Goldschmidt (1948). Edwards (1924) has described the sight as follows:

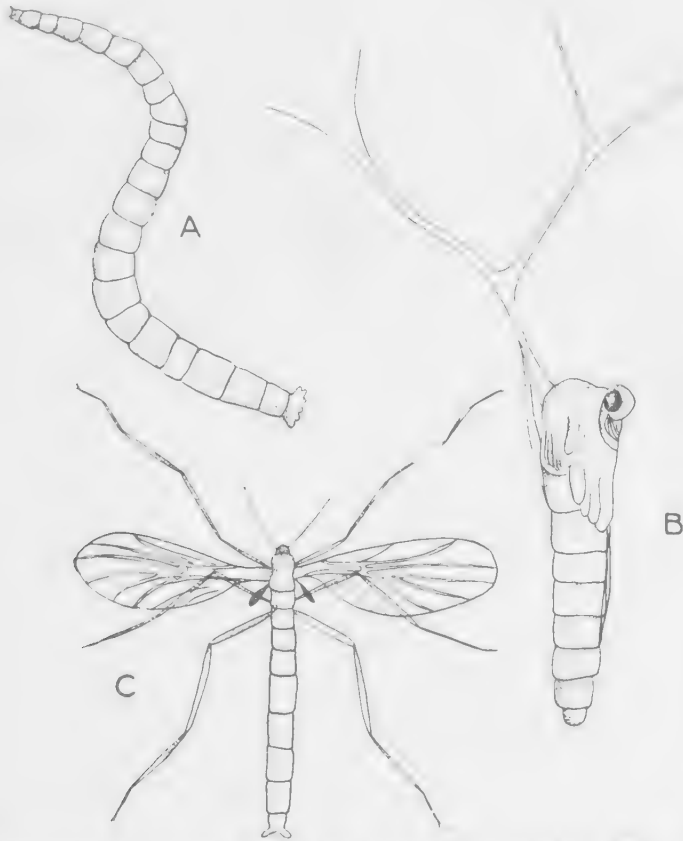


FIG. 125. *Bolitophila luminosa*, larva (A), pupa (B) and adult (C). After Hudson.

"Our guided wanderings (all the while deep underground) brought us at last to the edge of a pool. Here the guide made a speech about the glow-worms which adorned the roof over the water, pointing out the long glistening threads of a cobwebby texture let down singly by each tiny worm apparently to catch minutiae on the wing. . . . Vibrations of air carried by talking or any other sound affected the larvae which thereupon put out their lights. . . . And now, after due admonitions (to keep quiet) and obeying the order to leave all maps behind, we stepped cautiously in single file down, down to a still lower level.

... Then gradually we became aware that a vision was silently breaking on us. . . . A radiance became manifest which absorbed the whole faculty of observation—the radiance of such a massed body of glow worms as cannot be found anywhere else in the world, utterly incalculable as to numbers and merging their individual lights in a nirvana of pure sheen.”

Biology. The eggs are laid in a mucous glue on the ceiling and sides of the cave. The larvae which hatch spin a silken sheath from which the animals hang suspended by glutinous threads, often 6 in. to 2 ft long. They are carnivorous, eating small insects which are believed to be attracted by the light and become caught in the web, as noted by Norris (1804). Hudson had originally thought that the light did not serve to attract food but “to assist the larvae in escaping from enemies, as when disturbed they nearly always gleam very brilliantly for a few seconds, suddenly shutting off the light and retreating into the earth.”

Although the larval light is visible most of the time and is brightest on warm dark nights, it may be quenched, i.e., it is extinguished rather than hidden. The pupal light is intermittent, and the adult females are luminous for about two days after emergence. The male larva loses its luminescence about two days before emerging and the male adult is non-luminous.

Histology. The luminous organ of living *Bolitophila* can be clearly seen in the dilated terminal (eleventh abdominal) segment of the abdomen as four parallel luminous rods close together on the ventral surface of the slender rectum. The bluish green light is most intense from the dorsal surface. The ventral surface is covered with an ill-defined layer of possibly reflecting tissue. Wheeler and Williams (1915) first studied sections, using material collected in a tunnel near the Waitakari Forest, Auckland, New Zealand. The luminous rods are actually the dilated tips of the four Malphigian tubules. The “reflector” tissue appears to be “a syncytium made up of the hypertrophied and finely vacuolated tracheal epithelium, as it reveals no cell boundaries.” Large tracheal trunks enter the layer and break up into branches so that the luminous tips of the Malphigian tubules are well supplied with oxygen. The photogenic cells are filled with small granules similar to those of the fire-fly. The essential structure of the organ is evident from Fig. 126. In no other insects have excretory organs taken over a photogenic function although they have become modified for silk production in some forms. The New Zealand glow-worm can apparently control the light, turning it on and off, but the mechanism for doing this is unknown. Nerves to the luminous organ

have not yet been described. The light is certainly not bacterial in origin, but nothing is known of the biochemistry.

HYMENOPTERA

There are several instances of luminescent ants in the literature, some of them rather questionable. M. de Villiers (1842) reported to the Entomological Society of France that his gardener noticed a great number of luminous globules in a box of earth at Montpellier in 1837. De Villiers later examined the boxes and found them to be "inhabited by a large number of little ants of a yellow color; but it is not certain if the ants themselves or their nymphs possessed this phosphoric property." Apparently de Villiers did not see the luminescence, which

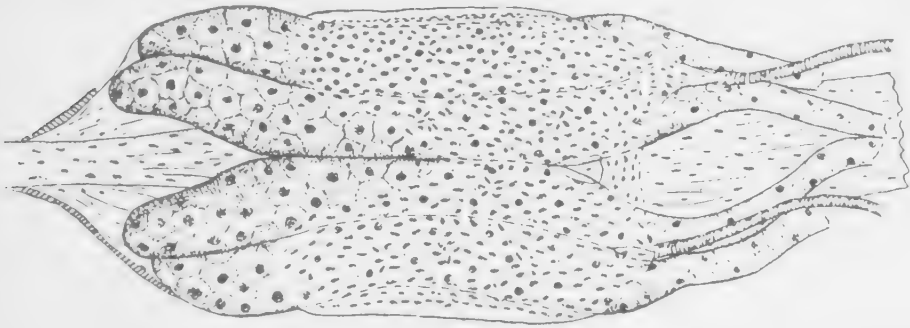


FIG. 126. The enlarged tip of the Malpighian tubules of *Bolitophila luminosa*, showing the luminous cells (stippled). After Wheeler and Williams.

may have come from the secretion of a luminous centipede or an earth worm in the box.

A much more definite case has been described by Ludwig (1902) from a letter of Mr. J. G. O. Tepper, State Entomologist at Adelaide, Australia. A friend of Tepper had found many examples of a large copper-colored diurnal ant, *Iridomyrex delectus*, to be more or less uniformly luminescent over the body and especially the legs. The light was observed at night and remained steady despite stimulation in various ways. This ant builds large nests on hard barren ground. Subsequent attempts to obtain luminous specimens failed although the nests were watered to bring the animals out.

A third and well authenticated case is the luminous ant found by G. P. Englehardt at 4,000 ft altitude on Mt. Lowe near Pasadena, California, and identified by Wheeler (1916) as a worker of *Camponotus maculatus*. The ant was noticed at night moving across a trail, caught and killed in a cyanide bottle, where the light "remained undiminished for fully five minutes, after which it decreased gradually and in fifteen minutes disappeared. The light issued ventrally from

the last two or perhaps three abdominal segments. It was greenish yellow, and very much like that of our common eastern fire fly, only it was constant, not intermittent." From the detailed descriptions of the last two cases the conclusion seems fairly certain that the light was due to infection with luminous bacteria.

Luminous wasps have been recorded by Schultz and Stern in their book, *The Ways of Fishes* (1948). Schultz wrote (p. 125):

"When I was in Venezuela I encountered another luminescent insect. . . . Some buzzing wasps came towards the [flash] light, and I grabbed my forceps and collecting bottle. . . . In my haste I dropped the flashlight, and it went out. But even in the blackness I was able to pick up three of the wasps with my forceps, for their abdomens glowed, and indeed continued to glow for a short time even after I had dropped the insects into the preservative." The preservative was 70% alcohol, and Dr. Schultz has written me the wasp was probably *Apoica pallida*, a nocturnal species with a white abdomen. Although observed alive by a number of entomologists, no other observations on its luminescence have appeared.

CHAPTER XIII

Coleoptera

INTRODUCTION AND CLASSIFICATION

Among beetles, members of the families Lampyridae, Phengodidae, Rhagophthalmidae, Drilidae, and Elateridae have developed the ability to produce light. They contain forms commonly known as fire-flies, glow-worms, lightning bugs, automobile bugs, cucujos, star-worms and railroad-worms. In addition, the early literature contains a number of questionable records of luminous beetles belonging to other families.

The best known of these is a member of the Paussidae, *Paussus* (*Edaphopausus*) *spheroцерus*, described by Afzelius in 1798. It dropped from the ceiling of his room at Sierra Leone at dusk and was placed in a box. Afzelius wrote: "One evening going to look at it, and happening by chance to stand between the light and the box, so that my shadow fell upon the insect, I observed, to my great astonishment, the globes of the antennae, like two lanterns, spreading a dim phosphoric light. This singular phenomenon roused my curiosity, and, after having examined it several times that night, I resolved to repeat my researches the following day. But the animal, being exhausted, died in the morning, and the light disappeared." The observation has never been confirmed. A luminous bacterial infection is possible.

Another beetle, widely quoted as light producing, is the bupestrid, *Bupestris ocellata*. Latrielle (1829) stated that the ocelli on the elytra were luminous. He also considered that two red oval spots covered with a downy membrane on the second abdominal segment of *Chiroscelis bifenestrata* perhaps indicated a luminous organ. Both these claims have been criticized by Percheron (1835), who stated that he had examined both males and females of *Chiroscelis* and they did not luminesce. Regarding *Bupestris ocellata* he said that the observations were incorrect and finally he held that another alleged luminous beetle, the *Scarabaeus phosphoreus* of Luce, described in 1794, was none other than the Italian fire fly, *Lampyrus* (*Luciola*) *italica*.

The older naturalists appeared to be eager to attribute phosphorescence to any unusual structure on an insect. *Bupestris ocellata* and *Chroscelis bilinestrata* are certainly not luminous, nor can luminosity be attributed to other yellow spots on beetles as claimed by Chandon (1880) for the carabid beetle *Scarities*. His words will be quoted to illustrate the casual method of designating luminosity. "These yellow spots are probably phosphorescent and luminous at night, but the opportunity to verify the fact has never occurred."

Another curious statement regarding luminescence connected with a carabid beetle has appeared in the literature. Reiche (1849), in a talk before the Entomological Society of France on "crepitation" of the bombardier beetles of the genus *Brachinus*, stated that their smoky discharge was luminous. The observation had been made regarding *B. africanus*, which produced "a phosphorescent luminous vapor in the darkness with an odor of chlorine." At the same meeting, M. Rouzet stated that he had seen a similar phosphorescent discharge from the *Brachinus* found under stones near Paris. Were the smoke of a bombardier beetle really luminous, the fact would be well known. Presumably the fine droplets of the discharge merely appear luminous in a dim light, like mist over meadows in the moonlight.

Finally, there is the recent report by Williamson (1948) of a dytiscid beetle which fell from a laboratory bench to the floor of the Malaria Bureau building in Kuala Lumpur, Malaya, and "Displayed a unique type of bioluminescence. The beetle appeared to emit bright flashes of white light, three or four at a time, from its eyes." An incandescent lamp was lighted at the time, and the phenomenon was probably a reflection of light, but Williamson never saw the flashes again, despite repeated observations.

Occasionally a beetle will be called luminous when it has attacked a luminous centipede and has been splattered by the luminous secretion. Such appears to be the explanation of a luminous *Goerius oleans* described by George (1851) at a meeting of the Entomological Society of London.

The following classification of Coleoptera by C. T. Brues and A. L. Melander¹ shows the relationships of various groups. Families containing luminous species are in italics. It will be observed that the Elateroidea are related to the Cantharoidea, containing the lampyrid-like beetles and that luminosity has appeared in both suborders.

Coleoptera

Adephaga

Caraboidea (8 families)

¹ *Bull. Mus. Comp. Zool.* 73, 1-672, 1932.

Gyrinoidea (Gyrinidae)

?Paussoidea (?Paussidae)

Cupoidea (Cupidae)

Rhysodoidea (2 families)

Polyphaga

Hydrophiloidea (2 families)

Staphylinoidea (12 families)

Cucujoidea (5 families)

Cantharoidea (12 families, including *Lampyridae* with *Rhagophthalminae*; *Cantharidae* with *Phengodinae* and *Telephorinae*; and *Drilidae* with *Karuminae*)

Mordelloidea (11 families)

Elateroidea (8 families, including *Elateridae*)

Dryopoidea (5 families)

Dascylloidea (11 families)

Histeroidea (3 families)

Colydioidea (30 families)

Tenebrionoidea (12 families)

Cerambycoidea (19 families)

Curculionoidea (12 families)

Scarabaeoidea (21 families)

CANTHAROIDEA OR MALACODERMATA

Fire-flies and glow-worms all belong to this group, strictly speaking to the *Lampyridae*, although the name has been applied to any insect which flies, like the elaterid beetles, or even to the New Zealand glow-worm, a dipterous larva. Interrelationships of the various families is uncertain. There appears to be no complete recent classification of the *Lampyridae* or the *Lampyrinae*, if this group is to be assigned the status of a subfamily. Consequently the classification² of the *Lampyridae* together with eleven related families, as given by Olivier in 1910, is presented. Genera and families with well-known luminous species are in italics, but some genera not italicized undoubtedly possess light organs, although data regarding luminescence is lacking. The relationship of families near the end of the list is not well understood and needs revision.

Lycidae (no luminous organs)

Lampyridae = *Malacodermidae*

Lamprocerinae (*Alecton*, *Pleotomus*, *Lamprocera*, *Phaenolis*, *Calyptocephalus*, *Psilocladus*, *Lucio*, *Dodacles*, *Dryptelytra*, *Aethra*, *Vesta*)

Lucidotinae (*Lucidota*, *Tenaspis*, *Lychnuris*, *Pyractomena*, *Lucidina*, *Lucernuta*, *Alychnus*)

Dadophorinae (*Dadophora*)

Photininae (*Cratomorphus*, *Aspisoma* = *Aspidosoma*, *Lecontea*, *Macrolampis*, *Heterphotinus*, *Photinus*, *Pentalacmis*)

Lampyrinae (*Microphotus*, *Lamprophorus*, *Diaphanes*, *Phausis*, *Pelania*, *Lampyris*, *Nyctophila*, *Phosphaenus*, *Phosphaenopterus*)

² From the *Coleopterorum Catalogus* (Vol. IX) of W. Junk.

- Megalophthalminae* (*Megalophthalmus*, *Harmatelia*)
Amydetinae (*Amydetes*)
Luciolinae (*Lampyroidea*, *Luciola*, *Atyphella*, *Curtos*, *Ototreta*, *Pteroptyx*,
Colophotia, *Pyrophanes*, *Bourgeoisia*)
Photurinae (*Pyrogaster*, *Photuris*, *Bicellonycha*)
Phengodidae (*Pterotus*, *Phengodes*, *Baloscelis*, *Zarhipis*, *Mastinocerus*, *Cenophengus*,
Trachelychus)
Karumiidae (*Karumia*, *Escalerina*, *Drilocephalus*)
Cantharidae or *Telephoridae* (no luminous organs), including *Tytthonyx*
Phleophilidae
Rhadalidae
Prionceridae
Rhagophthalmidae (*Diopoma*, *Ochotyra*, *Rhagophthalmus*)
Drilidae (*Drilus*, *Malacogaster*, *Paradrilus*, *Drilaster*, *Drilonius*, *Cerocosmus*,
Heliotis, *Selasia*, *Lemoglyptus*, *Stenocladus*, *Eugeusis*, *Anadrilus*, *Haplogeusis*,
Diplocladon, *Dodecatoma*, *Pachytarsus*, *Cyphonocerus*, *Cydistus*, *Phrixothrix*,
Telegeusis)
Malachiidae

In addition to the luminous genera of the Lampyridae indicated above, the following contain undoubted luminous species either in the larval or adult stage: *Pyrocoelia*, *Ellychnia*, *Pyropyga*, *Lamprohiza*, *Callopisma*, *Microdiphot*, *Presbyolampis*, *Diphotus*, *Jamphotus*, and *Erythrolychnia*. It will thus be seen that a high per cent of Lampyrid species are luminous and it is quite possible that luminosity has been overlooked in this and other families because it is restricted to larval stages, about which so little is known.

Lampyridae

The literature on fire flies, glow-worms, and lightning bugs far exceeds that of any other luminous group. Together with the "phosphorescence of the sea" they have excited the admiration of the poet as well as the scientist. They appear in the folklore and literature of all observant nations. Aristotle, Pliny, and the great encyclopedists of natural history described the "cicindula" and its habits in some detail. Among these books may be mentioned particularly Mouffet's *Insectorum sive minimorum animalium theatrum*,² published in 1634, and Aldrovandi's *De animalibus insectis*, published in 1638.

It is interesting to note that light from the egg of the fire-fly was known to Bartolin and mentioned in his *De luce animalium*, first published in 1643. Bartolin attributed the observation to Carolus Vinti

² See Cowan (1865), Hough (1901), and Joya (1912).

³ Translated in 1658 as "The theatre of insects."

⁴ Bartolin stated that Stephanus Spleisius also knew the egg was luminous.

millia of Palermo who also realized that winged fire-flies were males and wingless females, and that nature had "endowed them [the females] with a more vigorous light in order that they could call the males at night with their shine." Luminescence of the egg and use of the light as a means of attracting the sexes have been rediscovered any number of times by later students of fire-flies. The problem of the flying and the wingless glow-worms vexed the early naturalists even after Bartolin's book appeared and the relationship is sometimes confused today. Vintimillia was partly correct. A wingless fire-fly or a glow-worm may be an adult female of certain species in which the male is winged, but it may also be a larval form, either male or female, of a great many species of lampyrids.

Among the more or less popular articles, frequently dealing with the habits of fireflies, may be mentioned those of Guilding (1834), Gosse (1848), Acheta (1850), Joseph (1854), Fripp (1866), Anonymous (1880), Butler (1895), Stern (1897), Boyer (1897), Acloque (1905, 07), Floricke (1908), McDermott (1910, 48), Fabre (1914), Anonymous (1917), Wigglesworth (1949), and Peattie (1949).

It is difficult to say when the modern study of fire-fly luminescence begins. Some of the older investigators carried out extensive and often valuable experiments to determine the cause of the light. Among these may be mentioned DeGeer in 1755 and Gueneau de Montbeillard in 1782 on habits and metamorphosis and Förster in 1782, Beckerhinn in 1789, Spallanzani in 1796, Carradori in 1798, Macaire (1821), Dodd (1824), Carrara (1836), and Matteucci (1843, 47) on general physiological and biochemical observations. These men laid the groundwork for more modern experimental study.

General scientific accounts of fire-fly luminescence will be found in most of the extensive entomologies, such as Kirby and Spence (1828), Burmeister (1836), Lacordaire (1838), Packard (1896), Henneguy (1904), Berlese (1909), Imms (1924, 29, 31), Schroeder (1925), Vogel (1927) in Schultze's *Biologie der Tierre Deutschlands* and Meixner in Kuckenthal-Krumbach's *Handbuch der Zoologie* (1933-36). Special books or papers have been published by Gadeau de Kerville (1881, 87), Seaman (1891), Packard (1896), Bongardt (1904), Blair (1915), and others.

All general works on bioluminescence have also treated the lampyrid fire-flies in some detail, particularly Mangold (1910), Dahlgren (1917) and Maloeuf (1937, 38). By far the most critical and comprehensive and also the most recent monograph is that of Buck (1948), who has for many years made a special study of all aspects of fire-fly light production.

The true fire flies are common in almost all parts of the world, although rare in some regions like the Pacific coast of North America, certain islands and most desert countries.

Fossils. The remains of lampyrids of the oligocene or miocene have been found in Prussian amber by Berendt (1845) and are also mentioned by Menge (1856). A very well preserved fire fly with wings has been figured by Bachofen-Echt.⁶ *Luciola extincta* was described by Heyden (1861), and a fossil *Lampyris orciluca* by Heer (1865), who remarked that "this light beetle corresponding to our *Johanniswürmchen* undoubtedly diffused its pale light on summer nights of the miocene like its cousins at the present time." There are a few other records. Scudder (1895) mentioned a *Lampyris* sp. from the miocene of Oeningen, Baden, and a *Lucidota prima* from Florida miocene is listed in C. W. Leng's Catalogue of the Coleoptera of North America north of Mexico, published in 1920.

Morphology. Not all genera of the Lampyrinae are luminous. In some of them (*Ellychnia*) the adults are diurnal and have vestigial spots on the last abdominal segment, which are said (McDermott, 1948) to be faintly luminous a few hours after emerging from the pupa. In *Lucidota atra* and *Pyropyga fenestralis* the larvae are luminous but not the adults, which have also become diurnal in habit. Their eyes are small, unlike the nocturnal forms.

In some cases females are luminous and not males, but the author has not heard of luminous males and dark females. If both male and female can fly, the males usually have larger light organs than the female, and the sexes find each other by signalling. If the female is apterous like the European glow-worm, *Lampyris noctiluca*, the male has the less brilliant light organ and is attracted to the female by her long-lasting light. It is true in most species that the form of the lantern varies in the two sexes. In this respect lampyrids differ from elaterids, as there appears to be no sexual dimorphism in light organs of *Pyrophorus*.

In the North American *Photinus marginellus* and *P. scintillans* the females have only partially developed wings while the males are fully winged. The antennae are simple slender affairs in the luminous lampyrids, whereas in some non luminous species, like the North American genus, *Polychasis*, the antennae are pectinate.

The eyes of fire flies are particularly large, and Gorham (1880) has pointed out a definite relation between the size of the eyes of the males and the luminosity of the light organ of the female. The

⁶ A. Bachofen-Echt, *Der Bernstein und seine Einschlüsse*, Wien, 1949.

plumosity of the antennae is in inverse proportion, i.e., genera with plumose antennae usually have small eyes in both sexes.

In northern and central Europe the most famous lampyrid is the glow-worm of song and story, the female of *Lampyrus noctiluca* or *Lampyrus* (*Phausis*) *splendidula*, called "ver luisant" by the French and "Glühwürmchen" by the Germans. In both these forms the female is wingless. The *L. noctiluca* female has two great light plates on the fifth and sixth abdominal segments and four smaller ones on the fourth and seventh segments. The *L. splendidula* female has one large light organ on the sixth segment and thirteen small spots on the sides or center of the other segments. The males are winged and called Johannisikäfer in Germany. The *L. noctiluca* male has two small light organs on the last abdominal segment, and *L. splendidula* has two large organs on the fifth and sixth abdominal segments.⁷ Vogel (1915, 27) and Verhoeff (1924) have given an excellent account of the biology of these forms, which have been studied by a host of workers. There is also present in Europe a small fire-fly, *Phosphaenus hemipterus*, whose males have short elytra and the females are wingless.

In Italy and southern Europe the fire-flies belong to the genus *Luciola*, *L. italica*, *L. lusitanica* and *Phausis*, *P. Delarouzei* and *P. Mulsanti*. Both male and female are winged, but in *L. italica* the female's wings are shorter and she flies only occasionally. The light organs cover the fifth and sixth (last) abdominal segments in the male and are restricted to two small spots on the fifth segment of the female. Emery (1884-87) has made a special study of these forms and a most detailed monograph on the anatomy has been published by Bugnion (1920, 29).

In Japan also, the common fire-flies are of the genus, *Luciola*, *L. vitticollis*, *L. parva*, *L. cruciata*, and others, studied by Tagaki (1934), Okada (1935), and Hasama (1939-44). *Pyrocoelia rufa* has also been a favorite for investigation.

In North America and the Caribbean region the genera *Photuris* and *Photinus* contain the most species. Their light organs are on the ventral surface of the sixth and seventh abdominal segments. In some species like *Diphotus* the eighth may be the luminous segment as it is in larval forms of *Photinus* and *Photuris*. In other species such as *Lamprorhiza splendida* all ventral segments may contain luminous spots. In *Pyroctomena* the organs are lateral rather than ventral. All aspects of light production in these North American and Caribbean species have been studied by a large number of workers whose work

⁷ According to Vogel (1922) the lanterns are on the sixth and seventh segments as the first segment becomes fused with the thorax and inconspicuous.

will be referred to under various headings. Some of the different species of fire flies are shown in Figs. 127 and 128, and the various forms of light organs are shown in Fig. 129.

Eggs and Embryology. In addition to Bartolin in 1643, already mentioned, luminescence of the eggs of lampyrids have been noted by Kratzenstein in 1757, Gueneau von Montbeillard in 1782, Schmidt (1803), Strickland (1834), White (1835), Fennel (1835), Mulsant (1862), Jousset de Bellesme (1871), Laboulbene (1882), Dubois (1886),



FIG. 127. Japanese autumn fire-flies (*Pyrocoelia*). Left, larvae. Upper right, males. Lower right, females. After Watasé.

87, 1913), Lucas (1888), Dimmock (1889), Morley (1896, 1901), Priske and Main (1911), Main and Priske (1912), Fabre (1913), Williams (1916), Gerretsen (1922), and Raj (1943). A few, like Newport (1857) and Wielowiejski (1882), thought the eggs were luminous because of luminous material adhering to them, but this idea is not correct, for Owsianmikow (1868), Bongardt (1903), and many others have shown that the light is intrinsic. Hess (1920) has observed that the egg of *Pyropyga fenestralis*, whose adult is non-luminous, is slightly luminous when first laid and the larvae are also luminous. As development of the fire-fly egg proceeds, the light becomes concentrated in one place and periodic in appearance, according to Gerretsen's observations on *Luciola vittata*.

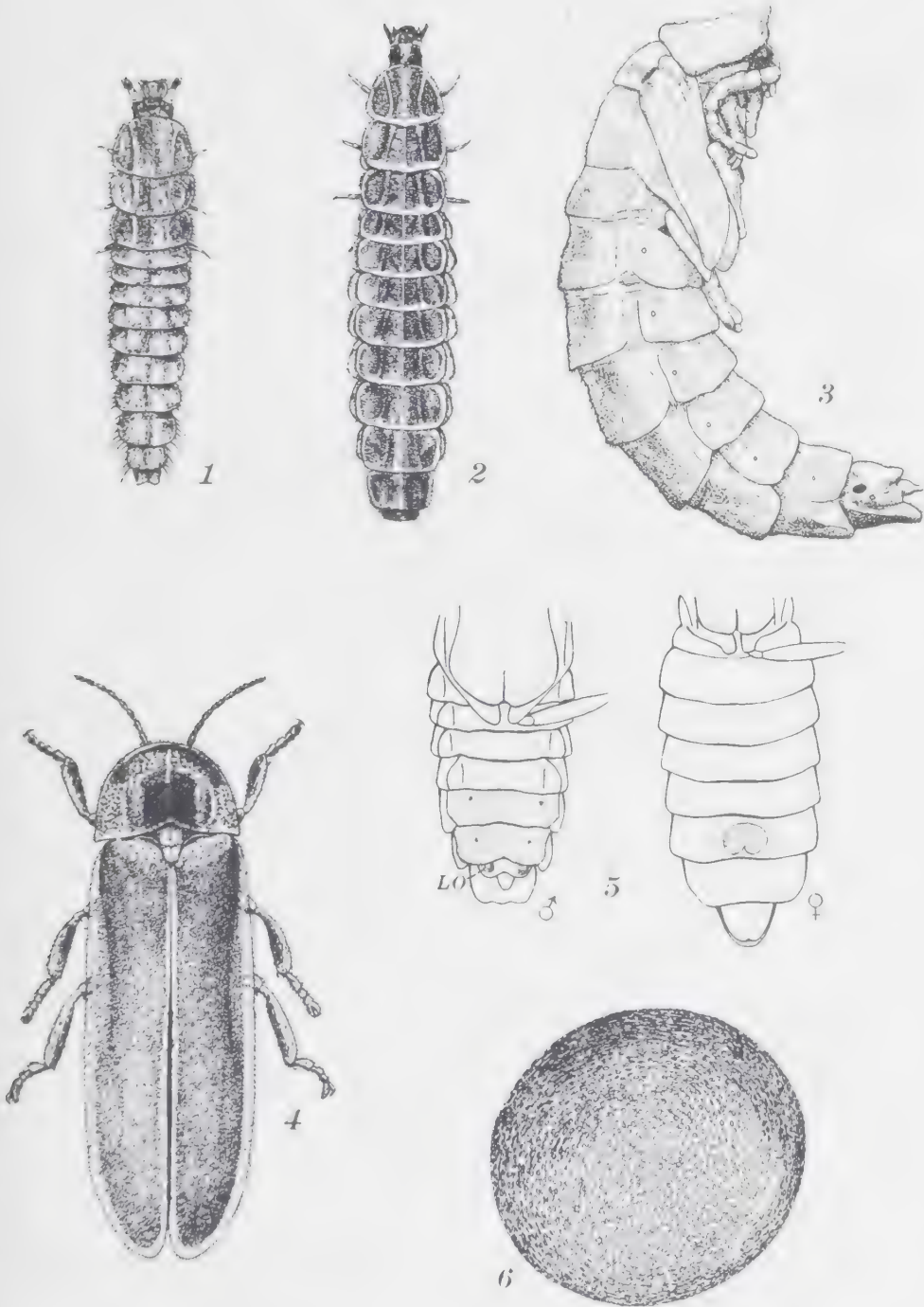


FIG. 128. *Photinus consanguineus*. 1, larva, first instar; 2, full-grown larva; 3, pupa; 4, adult male; 5, ventral view of male (left) and female (right) abdomens showing larval light organs (LO) and adult light organs (stippled); 6, egg. After Williams.

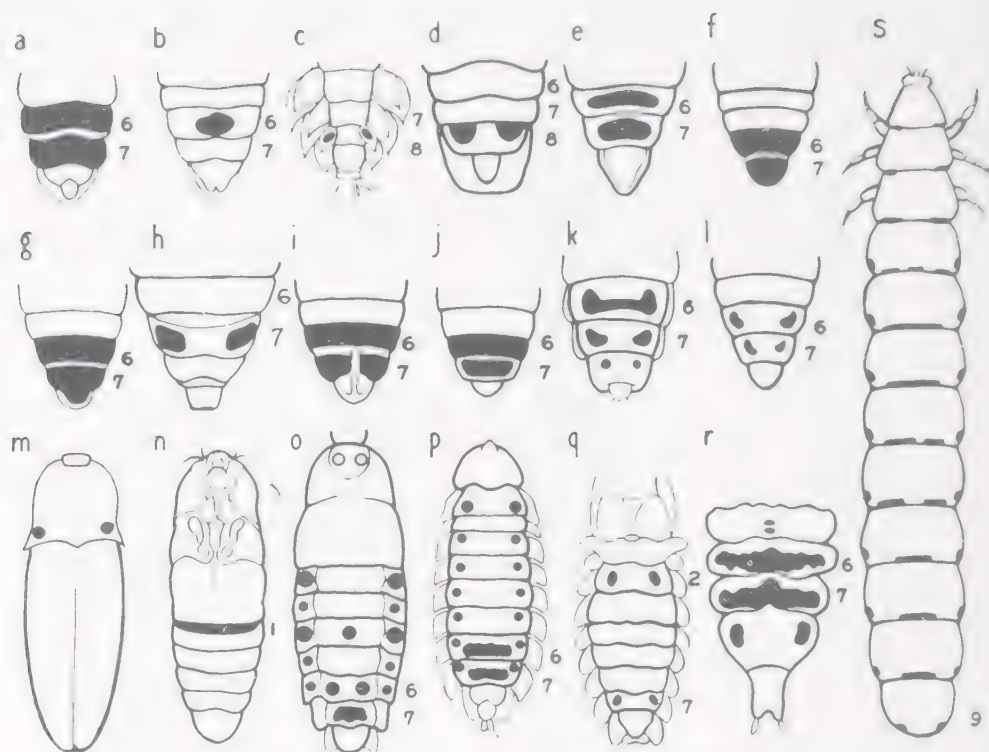


FIG. 129. Outline diagrams showing positions and sizes of photogenic organs in representative fire-flies. Photogenic organs indicated in solid black. Numbers refer to abdominal segments. Ventral views, unless otherwise noted. Sizes are variable and are not shown to scale, but normal size of *Phengodes* (s) is about 40 mm, *Pyrophorus* 25 mm, entire "average" fire-fly 10 to 15 mm. a, *Photinus scintillans*, male; b, *Photinus scintillans*, female; c, *Photuris pennsylvanica*, larva; d, *Diphotos montanus*, male; e, *Photuris pennsylvanica*, female; f, *Luciola chinensis*, male; g, *Luciola cruciata*, male; h, *Luciola lusitanica* female; i, *Luciola* sp., male; j, *Luciola lateralis*, male; k, *Pyrocoelia rufa*, female; l, *Leconicea lucifera*, female; m, *Pyrophorus noctilucus*, dorsal; n, *Pyrophorus noctilucus*, ventral; o, *Lamprorhiza* (*Lampyrus*) *splendidula*, female (the number of lateral organs is quite variable); p, *Phausis mulsanti*, female (the number of lateral organs is variable); q, *Phausis Delarouzei*, nymph, dorsal; r, *Lampyrus noctiluca*, female; s, *Phengodes* sp., dorsal. Figures a, b, c, and e after Hess. Figures f, g, i, j, k, and l, after Okada. Figures b, h, p, and q, after Bugnion. Figure o, from various sources. Figure r, after Bongardt. Figures d and s, after Buck. Figures m and n from Seaman. After Buck.

For details of the development of the light organs the reader is referred to papers by Vogel (1913), Dubois (1898, 1913), Williams (1916), Dahlgren (1917), Hess (1917, 22), Okada (1935), and Hasama (1944). Early workers suggested a number of tissues as giving rise to the lantern, basing their ideas on a study of the adult. Embryological investigation has led to the belief that either hypodermis

Dubois) or the fat body cells (Vogel, Williams, Hess) produce the light organs.

However, a distinction must be made between development of larval and adult organs, since the adult lantern is an entirely new development taking place during the pupal stage when the larval organs are still present and functional. Later the larval organs degenerate and disappear. Dahlgren (1917) was inclined to believe the adult organ arose from proliferation of the ventral hypodermis but Vogel, Williams, Hess, Okada, and Hasama agree that both the adult and larval organ arise from differentiation of fat body cells.

The events are described by Hess for *Photuris pennsylvanica* as follows:

"The first indication of the formation of the light-organs, in the embryo, is noticeable at the age of fifteen days, just as the embryo evolves from its backward-turned position and starts to coil up. At this time groups of fat cells, with their large globules which are colored dark by osmic acid, migrate ventrally in segment eight and come to lie in the region of the future light-organs. These undifferentiated light-organ cells are now continuous with the groups of fat-cells dorsal to them. As soon as the fat-cells become localized in the region of the future light-organs, their dark-colored globules become smaller in size and fewer in number. In fact, in the fifteen-day embryos there appears to be a gradual graduation from the cells lying next to the hypodermis, which contain smaller and fewer of these globules, to the fat-cells near the central part of the body, which contain more and larger globules. In the sixteen- and seventeen-day embryos, the light-organs are regular in outline, and they have become separated from the other fat-cells. The fat-globules are now smaller and fewer in number than on the fifteenth day. All cells that compose the light-organ are apparently now of the same histological structure. At the age of twenty days there begins to take place a differentiation of the cells of the light organs into the photogenic and reflector areas. At the age of twenty-two days the light-organs become functional and appear as six minute spots of light. The larvae emerge on about the twenty-sixth day of incubation.

"These larvae require nearly two years (about twenty-two months) to reach maturity, at which time they pupate. In mature larvae, about one half day before pupation, the cells of the fat-spheres, which lie near the hypodermis in the ventral part of the sixth and seventh abdominal segments, are liberated and become distributed along the hypodermis. These cells contain numerous fat globules, which appear dark after treatment with osmic acid. The fat-cells, which are

liberated from the fat-spheres during the last day of larval life and the first one or two days following pupation, compose a layer about three cells deep above the hypodermis. . . . The undifferentiated cells of the light-organs, at this stage, are all of the same general histological appearance, which suggests a common origin. The cells of the photogenic and reflector layers, in the five day pupae, are clearly differentiated. At this time the cells of both layers still contain some of the dark colored fat-globules. Tracheal epithelium, by the rapid division of its cells, now extends from the region of the body cavity down between the cells of the light-organs at regular intervals. It later gives rise to the trachea of the light-organs, together with their tracheal end-cells and tracheoles. Shortly before the light organs become mature, in both the embryo and the pupa, the fat-globules disappear and the organ takes on its characteristic adult structure. The light-organs of both the larva and the adult are formed from fat cells which become differentiated into the photogenic and reflector layers of the mature light-organs. Hence the light-organs are entirely mesodermal in origin."

Larval stages in development of *Lampyris noctiluca* and the natural history of the insect have been carefully studied by many observers. Newport (1857) left an extensive posthumous manuscript of careful observations, published by Ellis. Among other facts he also noted that the larval development took nearly two years. Priske and Main (1911) and Main and Priske (1912) have published excellent photographs of larval stages while Hasama (1943) has made a special study of the larvae of the Japanese *Luciola cruciata* and *L. lateralis*, and Ra (1932, 43) of Indian forms.

Larval stages of American lampyrids are fairly well known through the painstaking work of Williams (1917), Hess (1920, 22), and Balduf (1935), but space does not permit the recording of this work. Occasionally a larva with pupal or imaginal characters will appear, such as the prothetelous larva of *Photuris pennsylvanica* described by Williams (1914).

An unusual wingless glow-worm from 3,000 meters altitude in Ecuador has been described by Barber (1923), but the genus and species are uncertain; it is possibly *Phaenolis abditus*. The luminous organs are "two bodies protruding from the articulation between the penultimate and the antepenultimate body segments."

Aquatic Fire-Fly Larvae. The existence of a fresh water luminous mollusc, *Latia*, from New Zealand has already been noted. A second example of fresh water luminous animals is to be found among certain fire-fly larvae. The adult fire-fly is terrestrial, and the change of the larva to a water existence is no doubt recent, from an evolutionary

point of view. Special adaptation to aquatic life has occurred in at least two different ways—by means of a star-shaped funnel and tracheae, a sort of "Snorkel," and by means of tracheal gills—and a third form may use oxygen which diffuses through the chitinous body wall.

Ammandale (1900) first described aquatic glow-worms, which he observed in a small stagnant pond near Lampan, the chief town of Patalung, lower Siam. They were clinging, upside down, to plants floating at the surface of the water, and when removed to air the bluish light from the two small abdominal organs went out and only returned some time after they had been collected and replaced in a jar of water. The larvae were not silvery in color from air clinging to the body, and no special respiratory apparatus was observed. They never came to the surface to take in air. Probably oxygen diffuses into the larvae through the cuticle.

Six years later Ammandale (1906) observed another aquatic lamyrid larva in ponds near Calcutta among the roots of water plants. This larva was devoid of ordinary spiracles but possessed a star-shaped funnel at the rear end, supplied with large air tubes. The funnel could be extruded, pushed into an air bubble clinging to the plant to absorb oxygen and then withdrawn. It is probably the larva of *Luciola vespertina*.

The glow-worms with tracheal gills are of greatest interest. Blair (1927) received specimens found in a mountain stream near Djikoro, Bonthian, South Celebes, at a height of 4,000 feet. These larvae were under 2 ft of water and their chitin was thin and with little pigment, but they also possessed eight pairs of tracheal gill filaments projecting from the rear lateral corner of the first eight abdominal segments. The last segment was provided with a hooking device to cling to the surface of stones. The larval light came from the rear segments. They may have belonged to the genera, *Pyrophanes* or *Colophotis*.

About the same time, Okada (1928) described similar larvae from Japan. Two bluish luminescent spots were present on the dorsal lateral surface of the penultimate body segment. The larvae breathed by tracheal gills, never coming to the surface, but they pupated above water and all parts of the pupae emitted light. Two species of aquatic larvae were reared, one large, *Luciola cruciata*, and one small, *L. viticollis*. These larvae eat water snails, many of which harbor stages of the dangerous parasite, *Schistosomum*, which led Dr. K. Myajima to write a popular article in Japanese entitled "Protect fire-flies." Nevertheless Okada has bemoaned the fact that fire-flies are becoming scarce in his country.

Habits and Bionomics. The eggs of American fire-flies are laid on

or near the ground and hatch in about three weeks. The larvae differ considerably in habit. They live mostly in damp places among dead fallen leaves, becoming active at night and feeding voraciously on slugs and snails and small insect larvae. Photinus larvae are mostly burrowing and live a subterranean existence, whereas Photuris larvae live at the surface of the ground.

Fabre (1914) held that a substance like an anesthetic was injected by glow-worms into the snail to paralyze it, but Vogel (1915), Haddon (1915), and Hess (1920) showed that juices of the mid-intestine passed out through the hollow mandibles and partially digested the tissues before they were eaten. The larvae of American species rarely become active if the temperature is too low. They winter (in the case of most species which take two years of larval development) under stones or a short distance under ground, often in specially constructed chambers. Pupation is near the surface, in pupal cells. Knauer (1910) has observed lampyrid larvae on the snow, when they were known as "snow-worms."

Photuris adults are carnivorous, especially the females which kill and partially eat other fire-flies and insects placed at their disposal. They are very active and energetic fire-flies and have a disagreeable odor. Photinus species do not appear to eat in the adult stage and are relatively slow in their movements and less odorous. Space limitations prevent an account of the details of behavior of various species which will be found in the original literature.*

Use of the Light. Despite early designation of the light as a sex attraction, some have thought that it was a warning signal. There is the famous case of the Indian weaver bird (*Ploceus baya*) which builds a bottle-shaped nest and is supposed to collect fire-flies and stick them into bits of mud on the nest to frighten away bats, snakes, and other enemies (see Waterton, 1871; Severn, 1881; Dubois, 1885; Gadeau de Kerville, 1891). Although often repeated, this story is more sensational than true.

McDermott (1948) has reported that birds reject *Photuris pennsylvanica*, and Belt, in 1874, in his *Naturalist in Nicaragua*, has stated that monkeys do not eat them. Shelford, in 1916, in *A Naturalist in Borneo*, relates the story of a gecko that turned and fled when a fire-fly flashed just as the gecko was about to seize it. Although the larvae of

*The reader is referred to the papers of DeGeer in 1755, Maille (1826), White (1835), Gosse (1848), Joseph (1854), Newport (1857), Pergallo (1862), Wenzel (1896), Wasman (1896), Lodge (1903), Knab (1905), Steche (1908), Lund (1911), Bugmon (1919-29), Elmhirst (1912), Mast (1912), McDermott (1912-17), Pethen (1913), Haddon (1915), Hess (1920), Allard (1920), Gerretsen (1922), Cros (1924), Mehta (1932), Knapp (1939), Haneda (1939), and Hasama (1943).

lampyrids eat snails. Newall (1879) has reported finding a large snail that had eaten a glow worm, and he could see the light shining inside.

If fire-flies are distasteful, they might be mimicked by other insects, and there are in fact cases of similarity in color pattern," but the idea of the lantern of the fire fly as a means of defense or a warning signal is very dubious. The function of larval light organs and those of glow-worms might be to warn predators away, as Kaiser (1884) has held, but the possibility of attraction is just as probable.

The old discovery that the light is a mating device to attract the sexes is rather universally accepted at the present time. Nothing could be simpler than a fairly continuous light, like that of the female *Lampyrus noctiluca* to advertise her whereabouts to the flying male. This purpose has been recognized by Rogerson (1821), Dale (1834), Partitt (1880), Stillman (1880), and many others. In fact, Elmhirst (1912) found that the female *Lampyrus noctiluca* occupies a permanent position on the ground night after night and sometimes males are attracted to her in hundreds. Nearly all writers mention the fact that the lantern is held in the air so as to be easily seen. Curiously enough, in view of the greenish color of the light of the female, Elmhirst found *Lampyrus* males particularly phototropic to red as compared with blue or green or white light (a candle). Possibly the intensity of the light rather than the color complicated Elmhirst's experiments.

In those species among the lampyridae where both males and females are luminous, a fairly complicated signal system has been developed. This appears to have been first recognized for American fire-flies by Osten-Sacken (1861) and has been studied by McDermott (1910-17), Mast (1912), Hess (1920), and Buck (1935, 37). Each species of fire fly has a characteristic flash by which the female of one species can recognize a male of the same species and distinguish him from other species. Although a fairly continuous glow, like that of the female European glow-worm, would be the best method of attracting a male, at the same time the continuous light would also attract enemies, and this may have been a factor in the development of the flash.

The relation of the type of flash to the structure of the organ will be considered later, and only the type of flash as an adaptive means of bringing the sexes together will be discussed in one well-known case. According to McDermott and Mast the essential mating habits of

⁹ The lampyrid *Callophisma* and the lycid, *Thonalmus*, are similar in appearance but Darlington (*Trans. Roy. Entomol. Soc. London* 87, 681-96, 1938) doubted that this was a case of mimicry.

Photinus pyralis are as follows:¹⁰ "At dusk the male and female emerge from the grass. The male flies about, approximately 50 cm. above the ground, and emits a single short flash at regular intervals. The female climbs some slight eminence, such as a blade of grass, and perches there. She ordinarily does not fly at all, and she never flashes spontaneously, as does the male, but only in response to a flash of light, such as that produced by the male. If a male flashes within a radius of three or four meters of a female she usually responds, after a short interval, by flashing. The male then turns directly toward her in his course and soon glows again. Following this the female again responds by glowing and the male again apparently takes his bearings, turns and directs his course toward her. This exchange of signals is repeated, usually not more than five or ten times, until the male reaches the female and mates with her."

"Mast (1912) observed that males 'seldom if ever' respond to the flashes of other males, and he therefore concluded that they somehow distinguish between the flashes produced by the female and those produced by other males. He listed the following possible factors involved in this:

"(1) The female is always at rest when she glows while the male is ordinarily on the wing; (2) the form of the luminous area differs in the two sexes; (3) there is always a certain time relation between the glow of the male and that of the female in response to it; (4) the quality of the light produced by the two sexes may differ; (5) the intensity may differ; (6) the duration of the glow may differ. Mast maintains that he eliminated all these factors 'save possible difference in the duration of the glow.'"

Buck (1937) has continued the observations on the way in which the male distinguishes between male and female flashes and decided that such recognition depended on the time interval between the male flash and that of the female. This interval is 2 seconds at 25°, but does vary inversely as the temperature as described in the section on temperature. Males, in flying about, flash on the average every 5.8 seconds, but the female invariably responds to a male flash after 2 seconds. Buck found that only if another male flashed 2 seconds after the first male, would the first male fly toward the second male flash. A flash of artificial light irrespective of color and irrespective of duration (from $\frac{1}{50}$ to 1 second) would also induce a male to fly toward it. The color vision of both sexes of *P. pyralis* extended from some point between 520 and 560 m μ in the green to at least 690 m μ in the deep red. With a knowledge of the above facts, Mast, Hess

¹⁰ Quoted from Buck (1937), p. 412.

1920), Allard (1935), and Buck have all been able to attract fire-flies with flashlights, but Gates (1917) was not too successful.

Other species of fire-flies have other signaling systems and types of flash. The variation in type of flashing has been particularly studied by McDermott and is illustrated in Fig. 130. The exact type of flash also appears to vary in the same species in the same and different places.

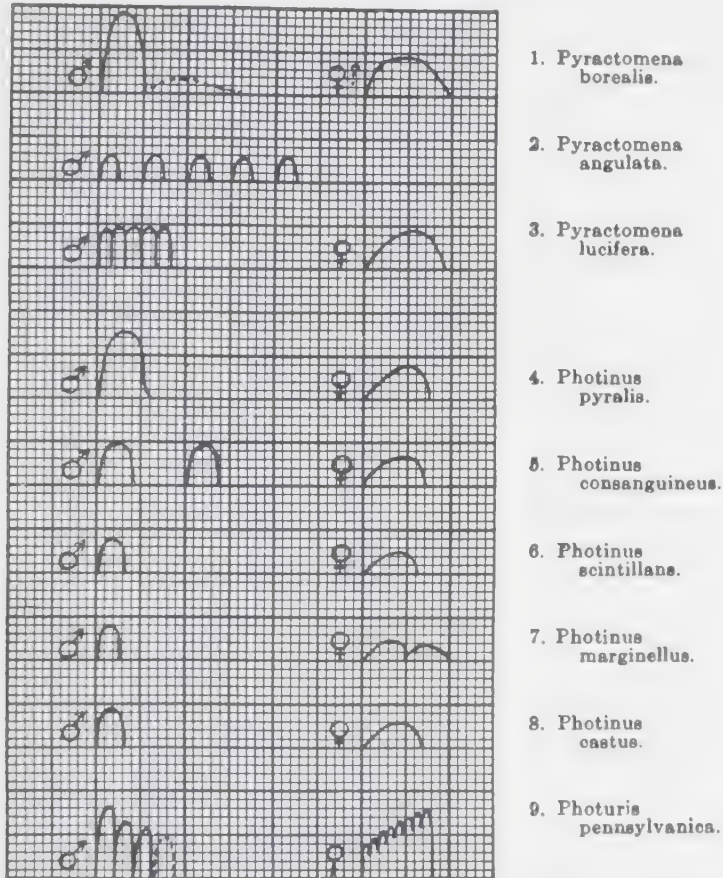


FIG. 130. Chart showing relative intensities and durations of flashes of American fire-flies. One centimeter vertically = approximately 0.02 candlepower; 1 cm horizontally = approximately 1 second. The flash of the males is at the left; that of females at right of chart. After McDermott.

McDermott (1948) has described three totally different types of flash among males of *Photuris pennsylvanica*. There are also slight differences in dimensions and coloring of this fire-fly correlated with the type of flash. As *P. pennsylvanica* extends from Massachusetts to Panama, it is quite possible that several different species or subspecies should be recognized.

Synchronous Flashing. It is quite obvious that a signal system in

which a female responds to a male after a definite time interval might lead to synchronism if a number of males responded to the flash of one female. McDermott (1916) felt that synchronism should occur but neither he nor Barber actually observed synchronism. However, synchronous flashing is occasionally seen in isolated groups of individuals among North American fire-flies but is definitely rare. Buck (1935) has induced it artificially.

Synchronism of an entirely different type is regularly found among tropical fire-flies of eastern Asia (Burma, Siam, Philippines) and the Indonesian Archipelago. In these regions all the fire-flies on one tree will flash, let us say, 100 to 120 times per minute with perfect synchronism, while on another tree some distance away the same synchronism will be apparent but out of step with those of the first tree. Early travelers in Siam (Kaempfer in 1727; Turpin in 1791; Goldsmith in 1810; Pallegroix in 1854; Bowring in 1857; Bennett in 1860; Cameron in 1865; Collingwood in 1868) were particularly impressed by the display which is one of the sights of the Far East. The mere records of such synchronous flashing are very numerous and will not be included in the literature of bioluminescence as they can be found in the bibliography of a review by Buck (1938).

Apart from the mere observation of the phenomenon little experimental work has been done. Every possible attempt to explain the synchronism has been made. It has been regarded as subjective, or an optical illusion, or the accidental starting of a group in unison. Some observers have held there was a pacemaker, with the synchronous response of the swarm to this leader.

According to Reinking (1921) the species responsible belong to the genus *Colophotia*. In Siam they accumulate on a special tree, *Sonneratia acida*, growing in low ground, often completely covered by water. Fire flies on the trees are all males, according to both Morrison (1929) and Smith (1935). The whereabouts of the female is unknown, but apparently the males fly from the jungle to the trees for the display. At this time the flashes are irregular, with no indication of the synchronism to follow. The absence of the female would appear to eliminate the phenomenon as a mating display, but further studies of the habits of this particular species of fire-fly should be made.

A remarkable aspect of the synchronous flashing is its persistence, keeping up according to Smith "hour after hour, night after night for weeks or even months, without regard to air currents, air temperature, moisture or any of the other meteorologic conditions which have been stated to influence firefly flashings; there may be a dead calm, a gentle breeze may be blowing or even a steady wind may prevail. The night

may be clear, the sky may be overcast or a light rain may fall without noteworthy effect on the rhythm or intensity of the flashing, but during bright moonlight the phenomenon is practically absent."

Morrison (1929) found that it was possible to inhibit the synchronism of a tree of fire-flies by exposing them to a bright light for about a minute and that when the light was turned off, the synchronism returned. The new synchronism arose from some individual or group located in the central part of the tree and extended over the entire tree in an irregular wave until all were flashing in unison again.

Most observers have not described a leader or pacemaker from which a wave of light passed outward and have frequently held that the trees are too large to allow a pacemaker to control the group. If the leader were in the center of the tree, a wave of light would not be as easily recognized as if the leader were at some point on the periphery. Alexander (1935) thought he could detect a leader and a wave of light that very quickly spread from tree to tree. This phenomenon occurred in the case of fire-flies collected on rows of mangroves along the Chao P'ya River, between Bangkok and the sea.

If animals tended to flash in a definite rhythm, which they do, and also if each immediately flashed when stimulated by a flash from another at a time near the natural period of the rhythm, all members would be soon brought to synchronism. Pacemakers might appear here and there (Richmond, 1930). The mechanism would be similar to rhythmic discharge of nerve cells and quite understandable, since the flash is nerve controlled in the fire-fly.

Histology. Investigation of the structure of fire-fly light organs has been carried out by more workers and in greater detail than in the case of any other luminous form. The mystery and fascination of an animal producing so bright a light without heat seems to have attracted many prominent zoologists to study the minute structure of the lantern in an attempt to elucidate its mechanism.

Most of the early workers described the more complicated type of light organ, which is made up of a ventral layer of pale parenchyma cells, the light producing layer, and a dorsal white "reflecting" layer of cells. The organ is richly supplied by tracheae and nerves whose distribution varies in different species and whose termination has been a source of controversy, not completely settled even today.

In Franz Leydig's (1857) *Lehrbuch der vergleichende Histologie des Menschen und der Tiere*, the fat body and the light organ of *Lampyrus splendidula* are described and figured. Leydig regarded the organ as a modified fat body but recognized that the light substance was different from fat globules. Kölliker (1857, 58) made one of the

best early histological studies. He pointed out that the organ was quite distinct from the fat body and made up of two layers. There was an albuminous substance in the lower layer and ammonium urate crystals in the upper layer. As all tests for the element phosphorus were negative, Kölliker regarded the light as resulting from oxidation of albumen by the nervous system.

Lindemann (1863) mistook the nerve ganglia for light organs and spoke of a "nervösen Apparate" and the luminescence of insects as an electric function like the electric organs of fish. This mistake was rectified by Max Schultze (1864, 65), who continued the histological study. He described the tracheal end cell in detail and made another important microchemical discovery. He noticed that these tracheal end cells stained markedly in osmic acid and suggested that they were the source of the light.¹¹ About the same time Tozzetti (1865, 70) studied the Italian form, *Luciola italica*, so that the fundamental structure of a variety of light organs was well understood by 1870.

The one great student of bioluminescence who neglected the fireflies was Panceri, as he devoted himself exclusively to marine forms. Nevertheless his influence is to be noted in the suggestion of Eimer (1872) that the tracheal end cells were comparable to the ganglion cells of *Phyllirrhoe* which Panceri (1872, 73) had incorrectly designated as the photogenic cells in this nudibranch.

Two men made important contributions regarding the air supply of glow-worms during the eighties. Wielowiejski (1882, 90) investigated the fine structure of the tracheal capillaries of *Lampyrus splendidula* and *L. noctiluca* and held that they did not end blindly in the light cells but anastomosed with each other to form an irregular net over the surface of the cells. He also pointed out that the tracheal end cells were not the source of the light, as Schultze had held, but were concerned with admission of oxygen to the "parenchyma" or photogenic cells, where a material is elaborated which undergoes slow oxidation under the influence of the nervous system.

Emery (1884-87) examined the light-emitting organ of *Luciola italica* microscopically and noticed that the light appeared in rings around the tracheal end cells. Like Schultze, he believed the tracheal end cells were the source of the light but that the "parenchyma" cells supplied the light substance.

The latter part of the nineteenth century saw a lull in the histological study of the fire-fly lantern, but research was again resumed in the twentieth century. Application of the most modern methods of study

¹¹ According to Buck (1948), Schultze said light began in the end cells although most workers quote him as believing that the end cells emitted the light.

was made by Bongart (1903, 04), Townsend (1904), Höllrigl (1908), Lund (1911), McDermott and Crane (1911), Vogel (1912-22), Geipel (1915), Williams (1916), Dahlgren (1917), Hess (1917-22), Bugnion (1919, 20, 29), Vonwiller (1920), Tagaki (1934), Okada (1935), Hasama (1942, 44) and Buck (1942, 48).

From the efforts of various workers on a great variety of species the latest information on histology is somewhat as follows. Buck (1948) has recognized six types of light organs in members of the Lampyridae, and related families, the Phengodidae (Phengodes) and the Drilidae

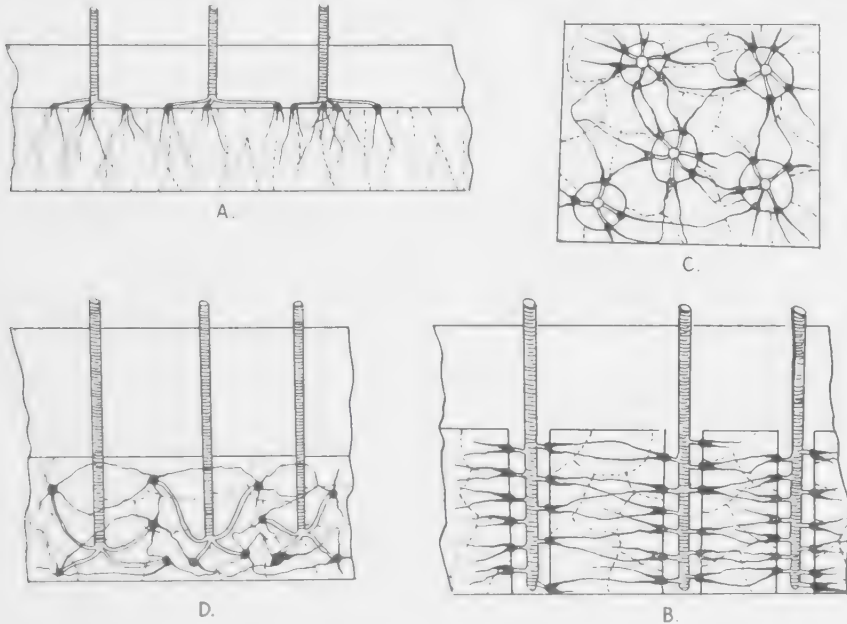


FIG. 131. Diagrams of the tracheal supply of three types of lampyrid light organ. A, type 4; B, type 6; C, same, top view; D, type 5. After Dahlgren.

(Phrixothrix). The first type, not found in the lampyrids but present in Phengodes, consists of masses of loose independent giant cells like oenocytes, without specific tracheal or nerve supply.

The second type, represented by the light organs of Phrixothrix, the lateral tuberculate organs of *Lamporhiza splendidula* (Wielowiejski, 1882; Bongardt, 1903) or the larva of *Phausis delarouzei* (Bugnion, 1929) is a compact mass of polyhedral photogenic cells with a tracheal supply but no reflector layer and no tracheal end cells.

The third type is similar to type two, except that a reflector layer is present. It is found in the larva of *Photuris pennsylvanica*, *Luciola cruciata* and *Pyrocoelia rufa* (Okada, 1935; Hasama, 1924) and the adult organs of *Diphotus montanus* and the female *Lampyris noctiluca*. The prothoracic and ventral light organs of the elaterid, *Pyrophorus*,

are of this type also. A drawing of a section of the larval light organ is shown in Fig. 132.

The fourth, fifth, and sixth types differ from the above in that they all possess tracheal end cells. They differ among themselves in the branching of the tracheae and the position of the end cells, shown in diagram in Fig. 131.

In type four, found, according to Dahlgren (1917), in "some forms of *Photuris*" the tracheae end in the tracheal end cells at the surface of the photogenic layer and tracheoles pass into the layer from above.

In type five, found in the Japanese *Luciola parva* and *L. viticollis*,

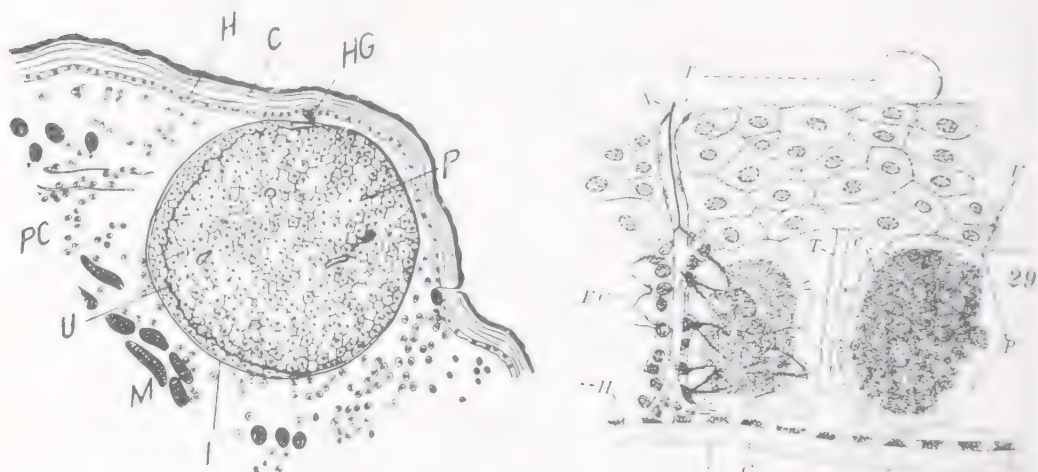


FIG. 132. (Left) Section of the light organ of a larva, *Pyrocoelia rufa*, showing photogenic cells, P, and urate layer, U. H, hypodermis; M, muscle; C, chitin; PC, phagocytes; HG, hypodermic gland. After Okada. (Right) Section of the light organ of an adult *Photinus consanguineus*, showing the photogenic cells, P; the urate layer, U; the tracheal trunk, T, with nerve N; the tracheal end cells, EC; hypodermis, H and cuticle C. After Williams.

the tracheal trunks branch among the photogenic cells, and the tracheal end cells are scattered here and there among the photogenic cells.

In type six, common among the American species of *Photinus* and *Photuris* and in *Luciola parvula* (Hasama, 1944) and *Luciola italica* (Tozzetti, 1870; Emery, 1884), there are large vertical tracheal trunks which penetrate both reflector and photogenic layer, forming "cylinders" of tissue in the latter. These cylinders are made up of regular short lateral tracheolar twigs, each with a tracheal end cell. In a surface view or horizontal section these cylinders are seen to form a regular triangular pattern with the tracheole branches and end cells forming a "rosette". The details of structure of type six can best be seen from the figures of the organs, reproduced in Fig. 132.

Tracheation. The light emission of fire-flies is dependent on an abundant supply of oxygen. Just as light organs of fish are richly supplied with blood through an extensive arteriolar and capillary systems.

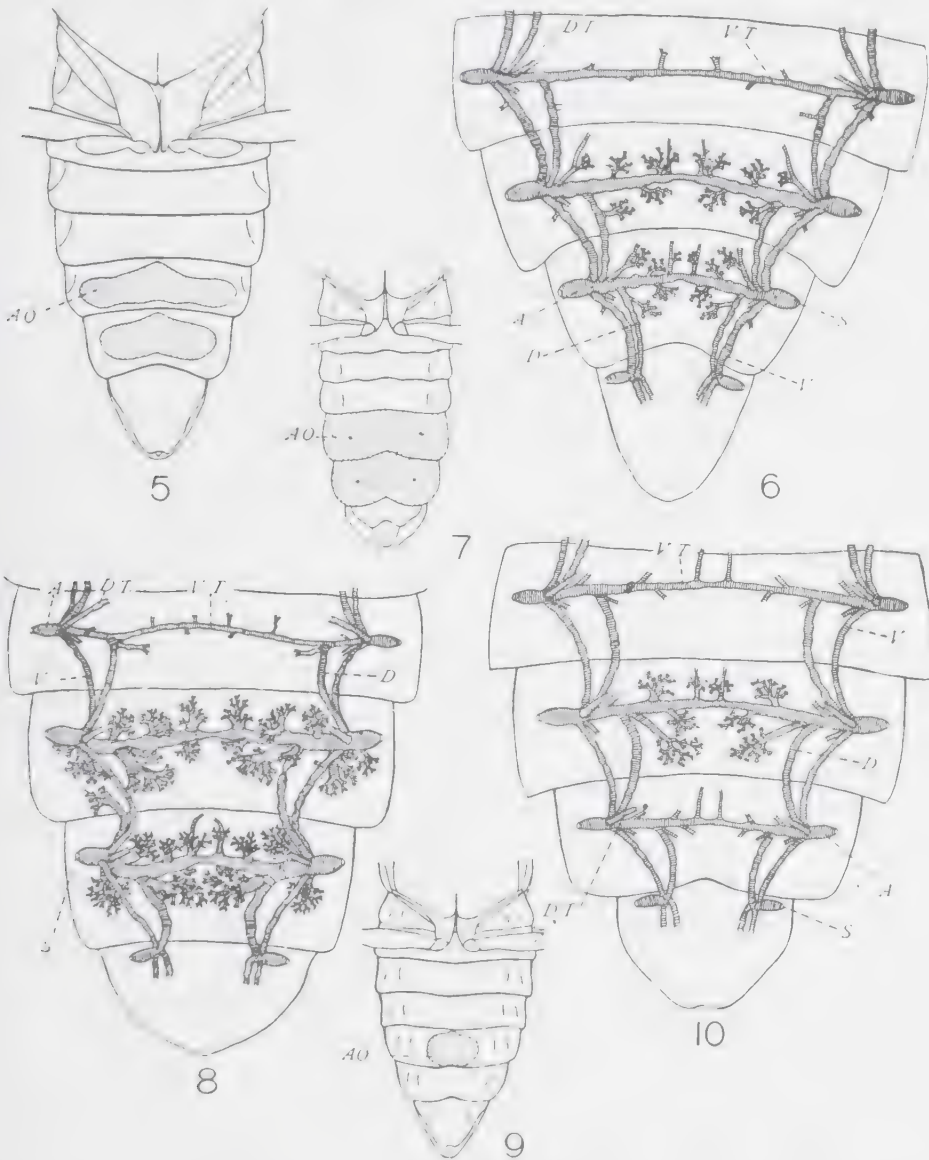


FIG. 133. Tracheal supply of abdominal segments of *Photuris pennsylvanica* female (6), *Photinus scintillans* male (8), and female (10), whose light organs are indicated in 5, 7, and 9, respectively. After Hess.

so the lantern of fire flies stands in special relation to the large tracheal trunks. This tracheation is particularly well shown in Fig. 133 reproduced from the study of Hess (1921).

Photogenic Cells. These cells are described as large and with hardly

visible boundaries, each with a single nucleus and, except for a peripheral zone near the tracheal cylinders, densely packed with minute granular or elongate bodies staining in acid or plasma dyes. Dahlgren's (1917) claim that the granules are spherical in the male, rod-shaped in the female fire-fly could not be confirmed by Hasama (1942) in *Pyrocoelia rufa* or by Buck (1948) in *Photinus pallens*. Vonwiller (1921) found that the granules stained like mitochondria, but Tagaki (1934) observed mitochondria in addition to granules in *Luciola cruciata*. However, there is little doubt but that the granules, like those in so many other luminous cells, are the source of the light.

Bacterial Symbiosis. Whether these granules are rod-shaped or spherical they resemble bacteria to a certain extent. Probably the first to suggest that fire-fly light might be due to luminous bacteria was Kuhnt (1907), by analogy with the bacteria of leguminous root nodules, but Knauer (1910) or Meissner (1907) were unable to agree with this idea. As early as 1914, Pierantoni was struck by the similarity in position and structure of the light organ of the larva of *Lampyrus noctiluca* and the mycetome organs of *Aphrophora*, an hemipterous insect which contains symbiotic organisms. He claimed to have demonstrated bacteria in sections and to have isolated bacteria from both the egg and the light organ of *Lampyrus*, but his cultures were not luminous. His "bacteria" in sections are very different from the granules and look like the mitochondria described by Takagi (1934) in *Luciola cruciata*. Vogel (1922) and Hasama (1942) were unable to grow luminous bacteria from the light organs of fire-flies.

In this connection it is interesting to note that Pfeiffer and Stammer (1930) who worked on luminous bacterial infections of living insects, believed the fire-fly light to be a "Secretleuchten" and not bacterial. They were unable to infect *Lampyrus noctiluca* with *Bacterium hemiphosphoreum*, a luminous bacterium isolated from living luminous caterpillars, although mealworms and many other insects could be infected and made luminous.

The fact that the egg and pupa of fire-flies glow continuously is in favor of a bacterial light, but is not a crucial criterion. In order to study regeneration of the light organ, Harvey and Hall (1929) removed the two lanterns of the larva of *Photuris pennsylvanica* and reared the operated insect, which pupated and emerged with a normal adult lantern. One might argue that removal of the larval organs had removed all luminous bacteria, but it is possible that non-luminous strains were present and later moved into the adult light organ. Since this adult organ is a completely new structure formed during the pupal period, it seems probable that potentially photogenic cells, not necessarily from

the larval organs, go into its formation. In fact, in an unoperated fire-fly the larval organs persist and luminesce for a short time in the newly emerged adult.

Buchner (1914) was originally convinced that the fire-fly light is bacterial in origin, but his position was not so certain in the 1930 edition of *Tier und Pflanze in Intrazellular Symbiose*. The recent work of McElroy and Strehler (1949) in which photogenic materials have been isolated and manipulated chemically, a procedure not as yet possible with luminous bacteria, rather definitely disposes of the symbiotic theory of fire-fly light.

It is now quite certain that light is produced by oxidation of some material in the photogenic cell layer and that the oxidation is certainly intracellular. It is also certain that the light is under nervous control except in the egg and pupa stages, but the point of ending of nerve fibers—whether on photogenic cells or tracheal end cells or both—is not settled. The two most disputed points have to do with the function of the reflector layer and the function of the tracheal end cell.

Reflector Layer. The "reflector" or urate layer is opaque in fresh sections by transmitted light in contrast to the clear photogenic layer, does not stain with acid dyes like eosin, and the cells are packed with sphaerocrystals of urate, according to the microchemical investigations of Kölliker (1858), Owsjannikow (1868), Tozzetti (1870), Heinemann (1872, 73) in *Pyroplorus*, Bongardt (1903), and Lund (1911). Modern tests should be carried out on the isolated layer to make certain just which purin body is present. Lund found that the reflector layer was decidedly acid to litmus while the photogenic layer was very weakly acid. Its function has been usually considered to be protection of internal tissues against fire-fly light or reflection of light from the lower surface. From its structure, scattering of light rather than reflection is indicated.

Kölliker, Tozzetti, and Lund suggested that the urates were waste products of the photogenic reaction while Schultze (1865), Dubois (1886) in *Pyrophorus*, and Gerretsen (1922) thought the photogenic cells were actually transformed into reflector cells. Transition cells between photogenic and urate cells have been described by some (Kölliker, 1858; Hasama, 1942) and denied by others (Lund, 1911; Geipel, 1915). Weitlaner (1909, 11) believed the urates were oxidized with light production and held that urate increased in old individuals, but this has been denied by Townsend (1904), Lund, and Geipel. Okada (1935) reported a decrease in thickness of the photogenic layer with age but no increase in the urate layer. It must be admitted that at present the function of the reflector layer is not clear. It is absent

in type 1 and type 2 lanterns, but present in so many fire flies that a real use is indicated.

Tracheoles and Tracheal End Cells. The tracheal twigs from a main tracheal trunk enter the tracheal end cells and branch into two or more tracheoles which pass toward the photogenic tissue. As Schulze first demonstrated, when an intact fire-fly is subjected to osmic acid vapor, the end cells reduce it to black suboxide or metallic osmium particles. These cells thus stain first and by proper exposure their outline, not at all clear in ordinary sections, can be observed. Too great exposure results in blackening of photogenic cells also.

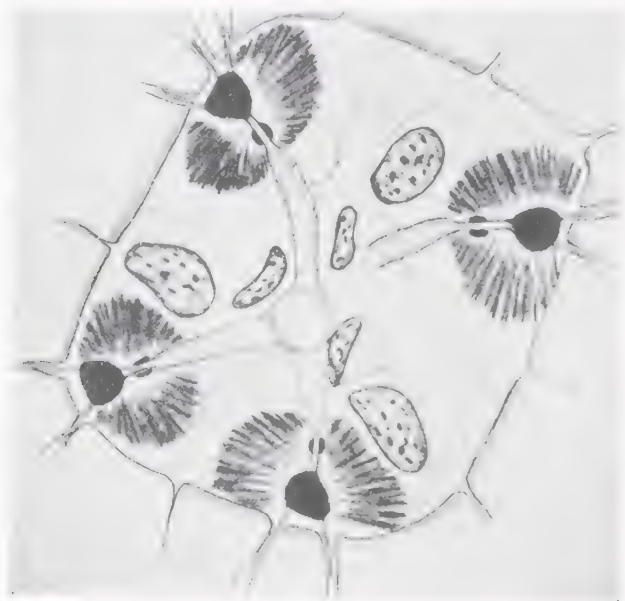


FIG. 134. Four tracheal end cells, showing possible valve mechanism. The radially arranged contractile fibers and black bodies which may be elastic structures are clearly seen in each cell. After Dahlgren.

Lund considered that there was a special reductase in the end cell involved in osmic acid reduction and in transferring oxygen to the photogenic cells. Both Bongardt and Dahlgren described a darkly staining annular swelling around the tracheal twig which Dahlgren considered to have sphincter properties, and he also observed "contractile radial fibers" in the cell. These are shown in Fig. 134. Both structures have played an important part in the theory of flashing which regards the tracheal end cell as a valve for admitting oxygen.

The course of the tracheoles in the photogenic tissue is a debatable point. The diameter has been given as around $1\ \mu$. Heinemann (1872) and Lund (1911) believed the tracheoles penetrated the photogenic cells, whereas most histologists including Buck, regard them as

merely running between the cells. Some workers describe them as ending freely while others have observed anastomoses, and Buck, by silver nitrate staining methods, has found an ultratracheolar network between tracheoles which lie parallel and close to each other. The tracheoles are probably filled with air, since liquid filled tubes would offer no advantage and in fact would retard the diffusion of oxygen to tissue. However, a line of demarcation between air and liquid cannot be established experimentally as the manipulation and preparation of tissue for microscopic examination is certain to introduce artificial conditions. The part played by fluid movement in tracheoles will be discussed later.

Nerve Supply. Nerves in the photogenic tissue were observed by the earliest workers, Kölliker and Schultze, but the termination has not yet been established with certainty. The nerves follow the tracheal trunks as in most insect tissue. The views of various histologists has been summarized by Buck (1948) as follows:

"Tozzetti and Emery found no connection between nerves and any sort of cells in *Luciola*. Kölliker and Schultze could not trace the finer nerves to their ends in *Lamprorhiza*, though Schultze thought it likely that they innervated the photogenic cells. Wielowiejski found direct connections between nerves and the surfaces of photogenic cells in *Lamprorhiza*, as did Owsjannikow (1868) in *Lampyrus*. Owsjannikow even described the nerve as penetrating to the nucleus, though Wielowiejski decried this idea. Geipel and Hasama (1942) reported that in *Photinus marginellatus* and *Pyrocoelia rufa*, respectively, the finest nerves connect directly with the end cells (to the nucleus, according to Geipel), and in *Lamprorhiza* and *Lampyrus*. Bongardt found them connected with the exterior of both end cells and photogenic cells.

"There is, therefore, no general agreement on the details of the nervous supply of the photogenic organ. In my opinion, a really convincing answer to this problem will require a full investigation which is devoted to this one point and makes use of modern neurological techniques."

Physiology. It is often difficult to separate anatomical from physiological studies on the fire fly, so closely is the function of the organ based on its structure. A similar connection exists between physiology and biochemistry. Under the heading Physiology, we shall be chiefly concerned with the control of the light. Most of the early workers remarked that control was under the "will" of the insect and ceased when the insect was injured or died but that the luminescence of the lantern material continued. The best example of the nervous control of the flash is to be observed when the animal is decapitated. Practically all

workers (Macaire, 1821; Peters, 1841; Verworn, 1892; Prowazek, 1908; Lund, 1911; Williams, 1916) have established the fact that flashing ceases immediately, although a dim constant glow may persist or appear later.

The first electrical stimulation of the animal appears to have been made by Macaire (1822), followed by most subsequent investigators who studied the physiology of light production. Todd (1826) has reviewed knowledge of the nature of lampyrid light. Among later nineteenth century workers the papers of Murray (1826), Peters (1841), Matteucci (1843, 47), Joseph (1854), Carrera (1836), Kölliker (1858), Owsjannikow (1868), Jousset de Bellesme (1880), and Verworn (1892) are of greatest interest. Some of the experiments on stimulation should be repeated with modern microelectrode technique, but there can be no doubt that there is reflex control of flashing and the neural pathways are similar to reflexes in other insects which involve light reflexes. Verworn (1892) described an automatic center in either the supra- or subesophageal ganglion of *Luciola italica*.

Among twentieth century students of fire-fly physiology the papers of Bongardt (1903), Steinach (1908), Kastle and McDermott (1910), Lund (1911), Gerretsen (1922), Brown and King (1931), Snell (1931, 32), Maloeuf (1937, 39), Buck (1937, 46, 48), Hasama (1939, 42, 44), and Alexander (1943) are important. Almost all are concerned with the mechanism of flashing and the influence of changed conditions on the flash. Steinach (1908) regarded light production as a form of response to excitation and showed that summation of subminimal stimuli could excite luminescence.

Correlation between structure and function is particularly well seen in presence or absence of the tracheal end cell, which is closely connected with the type of flash. Were these cells restricted to the light organs of insects we might unhesitatingly consider them to be a specific control mechanism for light production, but they occur in non-luminous insects and also, according to Wielowiejski (1882) and Geipel (1915) in fat-body and dorsal layer of fire-flies. However, these cells in non-luminous tissue look smaller and different from tracheal end cells in the photogenic layer.

Nevertheless there appears to be a definite connection between the complexity of tracheal end cell groupings and the brightness and short duration of the flash. Buck (1948) has classified methods of lighting into four groups: (1) the continuous glow, (2) the intermittant glow, (3) pulsation, and (4) the flash. The third and fourth are associated with the presence of tracheal end cells.

The continuous glow which lasts day and night is observed only in

the egg and pupa of lampyrids. It is also characteristic of the cream-colored adult female *Phengodes*, whereas the brown larval forms of *Phengodes* and larval forms of fire-flies (the glow-worms) exhibit the second type, the intermittent glow. The light appears slowly, rising for seconds to a maximum value which may persist ten to eighty seconds and then slowly fades out. The adult *Diphotus* and females of *Lampyrus* also exhibit the intermittent glow, as well as *Pyrophorus*¹² and *Phrixothrix*.

In pulsation, the series of light peaks, which may be 6 to 13 per minute in *Pyrocoelia rufa* or 60 to 110 per minute in *Luciola lateralis* (Hasama, 1942) are superposed on a background level of light, i.e., the light does not die out completely. It is possible that some of the rhythmically flashing tropical east Asian and East Indies forms belong in this category, while others may belong in the next group, the flash, which contains most fire-flies.

The flash rises from zero to a maximum intensity value in a fraction of a second and falls to zero again. Usually this is repeated at a time interval and for a series of flashes characteristic of the species, as illustrated in Fig. 130. Sometimes the flash will be single and very bright, lasting for approximately one second as in the Jamaican species, *Photinus pallens*, which the author found to be almost blinding. It can be compared to nothing less than a photographic flash bulb of long duration. Dr. Buck has assured me that *P. xanthophotis*, also from Jamaica, is even more brilliant.

It must be emphasized that the above types of lighting are based on normal uninjured animals. A lampyrid whose lantern is supplied with tracheal end cells may exhibit, in addition to the flash, a steady glow of the organ and also scintillation. These last two types of illumination often appear under abnormal conditions and have been important in analyzing the mechanism of the flash.

Time Relations of the Flash. Estimation by McDermott and others of the duration and time intervals between flashes have been made with a stop-watch. The development of photocells and the simple recording devices, such as the string galvanometer or cathode ray oscillograph, have made flash recording a simple matter. Records of *Photuris pennsylvanica* flashes were first obtained independently by Snell (1931, 32) and Brown and King (1931). These indicate very clearly the usually symmetrical nature of the response, having the general form of a distribution curve. Buck (1948) has calculated that in the lantern of *Photinus pyralis* there are 6,000 cylinders, each with eighty to a hundred end cells and each giving off two tracheoles which must supply

¹² Pulsations in *Pyrophorus* have been recorded by Harvey (1931).

15,000 photogenic cells. He observed that cylinders act as units in normal flashing, while Alexander (1943) observed in "scintillation" that one tracheal end cell flashed independently of other tracheal end cells. As these units could not be set off by nerve impulses in absolute synchronism, there is every reason why the flash should represent the timing difference of the units of the lantern.

At a temperature of 24°C the spontaneous flash of the male of *Photuris pennsylvanica* has the time relations shown in Fig. 135, lasting on the average 0.14 ± 0.03 second (Snell). With electrical stimulation of the thorax, the reflex flashes of males recorded by Brown and

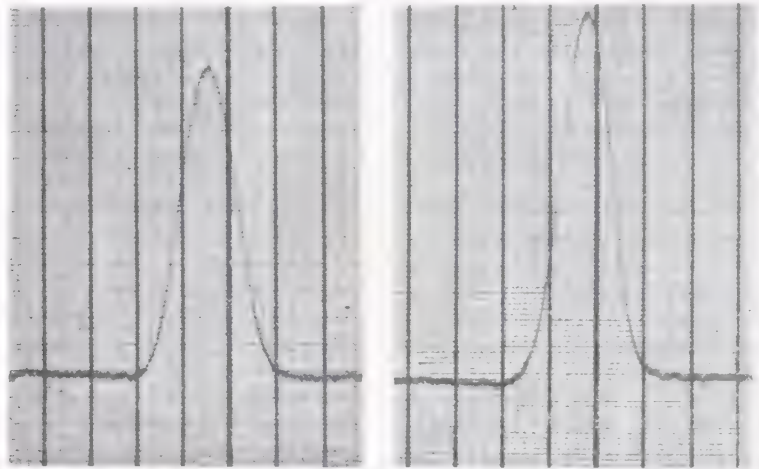


FIG. 135. String galvanometer records of the flash of *Photuris pennsylvanica*. Light intensity vertical and time between lines 0.04 second. After Snell.

King lasted on the average 0.15 second at 23°C. The female exhibits indication of the summation of two successive responses. The time between these responses and the magnitude of the second response are variable so that records differ considerably.

Both Snell (1931, 32) and Brown and King (1931) found the duration of the flash to be fairly constant while the intensity varied greatly. This is understandable on the observation of Lund (1911) and others that "either few or many discrete and definite individual areas of a luminous organ may be involved in any flash. The factor affecting the height of the curves but not the duration is the expression of the number of units of the luminous organ in action in that flash, the factor affecting primarily the duration is an expression of the activity of the controlling mechanism of these individual areas." The quotation is from Snell (1932), who studied the effect of oxygen tension on the flash and interpreted his results to mean that control of the flash was by admis-

sion of oxygen to the photogenic cells. He designated the tracheal end cell as the structure responsible.

Occasionally records in which the die-away portion of the flash was considerably prolonged and logarithmic in character over its whole length were obtained. They resembled the decay of light from an incandescent lamp after the current has been cut off or the decay of luminescence from a mixture of Cypridina luciferin and luciferase. The meaning of these unusual flashes was not ascertained. Most of the flashes exhibit die-away curves that are only approximately logarithmic.

Fire-fly flashes from two other species, *Photinus scintillans* and *P. pyralis* have been recorded by Alexander (1943), using a new type of circuit (Butt and Alexander, 1942) by which the flash could be photo-

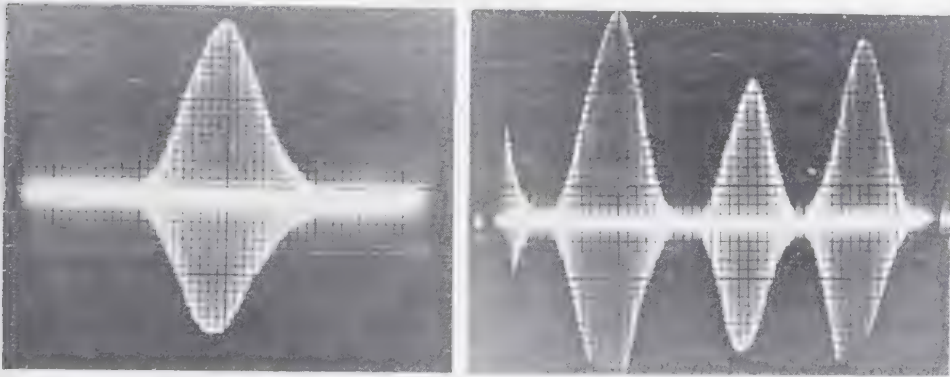


FIG. 136. Records of fire-fly flashes obtained by "A.C. bridge unbalance" method. Left, *Photinus pyralis* male at 26°C. Right, three flashes of *Photuris pennsylvanica* male at 29°C. Light intensity vertical; time between lines approximately 0.005 seconds. After Alexander.

graphed with an ordinary camera on a cathode ray oscillograph screen. The records, shown in Fig. 136, were essentially like those of *Photuris pennsylvanica*, but occasionally double flashes and prolonged flashes were observed, both in the records and in the field. The effect of oxygen tension and of increase of air pressure was studied in a further attempt to elucidate the mechanism of flashing. Like Snell, Alexander concluded that admission of oxygen to the photogenic cells by tracheal end cells controlled the flash.

Mechanism of Flashing. All observers agree that the normal flash is under nerve control but, as has been pointed out, it is not certain whether nerves end on photogenic cells, tracheal end cells, or both. If the old and incorrect ideas which connected flashing with breathing movements or with closure of spiracles¹³ and muscular contraction of

¹³ Buck (1946) found the spiracles normally closed and no spiracular activity connected with flashing.

the abdomen are excluded, three modern theories to account for the flash have been advanced.

The first theory regards the flash as similar to the stimulation of a *Noctiluca* or the photogenic cell of a pennatulid. Light emission is the response of the cell to excitation, whatever the means of setting off the light-emitting reactions within the cell. As in pennatulids the fire fly stimulus would be nervous, and light emission would be comparable to stimulation of a muscle. According to this theory nerves in the fire fly must end on the photogenic cells.

The second theory, particularly advocated by Dahlgren (1917), regards the flash as due to admission of a puff of oxygen to the photogenic cells by means of a valve mechanism controlled by the tracheal end cells. In order to control the valve mechanism, nerves must go to the tracheal end cells.

A third theory, proposed by Maloeuf (1938), postulates the control by movement of fluid in the tracheoles and is based on Wigglesworth's idea that osmotic pressure increase in a cell during activity withdraws water from the tracheole into the cell and allows a new supply of air, i.e., oxygen, to come in contact with the cell. According to this theory nerves must go to the photogenic cells, stimulating them to activity, i.e., light production, with consequent admission of oxygen when most needed. Maloeuf found that injection of hypertonic solutions into a fire-fly caused permanent intense glowing of the lantern, presumably by removing the tracheolar barrier to free diffusion of air, while markedly hypotonic solutions (tap water) led to death and cessation of light production in an animal which had been flashing.

Alexander (1943) was able to confirm Maloeuf's results with hypertonic solutions but not with hypotonic solutions. He found that injection of distilled water (instead of tap water, which may have been toxic due to its chlorine content) had no effect on the activity of the animal except to increase the rate and intensity of flashing. Moreover many injected solutions cause a continuous glow irrespective of their osmotic pressure so that the results with hypertonic solutions might have been due to an effect on the nervous system, especially as the lantern showed "scintillation" before the steady glow appeared.

There is some question as to whether Wigglesworth's original theory is correct, and observations of tracheolar water movement indicate that it is too slow to account for the rapid flash of a fire fly. The tracheolar movement theory applied to the fire-fly flash appears inadequate.

It is quite conceivable, in fact likely, that the first two theories are both correct—that the tracheal end cell admits more oxygen to the

photogenic cell at a time when it is most needed, i.e., when the photogenic cell is excited to luminesce by nerve stimulation. Double control is widespread in the functioning of vertebrate organs, for example the vasodilation which accompanies the stimulation of muscle or gland tissue. Its purpose is to increase the supply of oxygen and nutrients during the time of greatest demand. Whether a dual control occurs in the fire-fly or not, it is obvious that a study of the effects of oxygen tension on the flash will reveal important facts regarding the mechanism.

Relation to Oxygen. One of the first interests of early experimenters was to discover the necessity of air for various vital processes. Apparently the first person to study the relation of lampyrids to oxygen was Förster in 1782, who found the light to be extinguished in hydrogen and CO_2 . Spallanzani in 1796, Grotthus (1807), and Macaire (1821) obtained the same result, while Davy (according to Macartney, 1810) found no such effect. Impure gases containing traces of oxygen undoubtedly explain this last result. Carradori in 1798 held that the light remained in a vacuum, while Macaire found the light to disappear. The absence of light in absence of oxygen has been confirmed by Macaire (1821), Murray (1826), Matteuci (1847), Joseph (1854), Owsjannikow (1863), Jousset de Bellesme (1880), and so many others¹¹ that there can be no possible doubt of the fact. Although Kanda (1920) made a special study of the necessity for oxygen in *Luciola viticollis*, papers still appear (Shibita, Takeda, and Inoue, 1936) which hold that oxygen is unnecessary and that the light is due to triboluminescence.

Förster, Spallanzani, Macaire, Murray, and Matteucci found the light to become brighter in pure oxygen while Beckerhinn in 1789, Hermbstadt (1808), and Davy (according to Macartney) did not. The exact relation in pure oxygen has been carefully studied by Alexander (1943), who found an initial increase in light intensity followed by a decrease, which explains the contradictory results previously obtained.

As oxygen is necessary for luminescence in the fire-fly it *could* be the factor involved in control. Depending on whether control is by a valve mechanism or direct stimulation of photogenic cells, different oxygen tensions might be expected to act differently. The argument is as follows. Experiments on luminous bacteria and luminescent mixtures of Cypridina luciferin and luciferase have shown an independence of light intensity and oxygen pressure within wide limits (2—

¹¹ Arnold (1881) has claimed that *Lampyris noctiluca* will light when stimulated electrically in oxygen-free hydrogen, another instance of an impure gas.

760 mm in bacteria. There should therefore be little change in flash intensity with changing oxygen pressure, if photogenic cells are directly stimulated.¹⁵

On the other hand, if nerve stimulation of tracheal end cells opens a valve like device admitting oxygen to the photogenic cells, and assuming no effect of oxygen pressure on the duration of tracheal end cell valve opening, the lower the oxygen pressure the less oxygen would be admitted to the photogenic cells in one opening of the valve. Hence the total light in a flash should be more or less proportional to the oxygen tension.

A study of the flash in relation to oxygen pressure has been made by Snell (1932). Normal fire-flies (*Photuris pennsylvanica*) were placed in mixtures of oxygen and nitrogen varying from 4 mm O_2 to pure

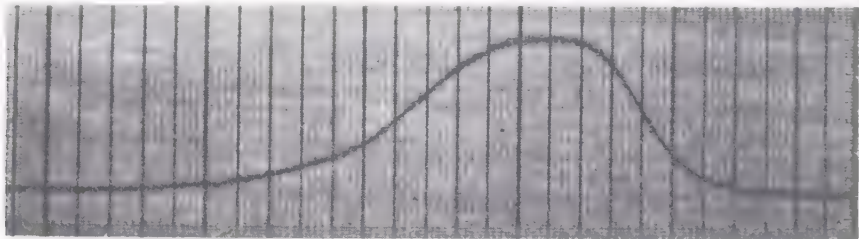


FIG. 137. String galvanometer record of the prolonged flash at 10 mm mercury oxygen pressure. Light intensity vertical; time between lines 0.04 second. After Snell.

oxygen, and records were taken of the spontaneous flash. It was found that at low tensions (below 4 mm) no flash occurred, but a faint steady glow of the organ, as if some controlling mechanism had opened to allow the small amount of oxygen to enter. Between 4 mm and about 20 mm the flash is much prolonged (see Fig. 137), whereas above 30 mm and up to around 300 mm the duration of the flash is quite constant. Above 300 mm O_2 a steady glow of high intensity appears, indicating that the flashing mechanism has been interfered with.

Since the intensity of a fire-fly flash under captive conditions is very variable, Snell was forced to select the most brilliant flashes at different oxygen pressures from among a large number of records obtained. When this was done, the intensity was observed to increase from 45 to 75 (in arbitrary units) between 40 mm and 150 mm O_2 , an increase of 60% while the O_2 pressure was increased nearly four times as shown in Fig. 138. Since the duration of a flash was constant over

A better comparison would be the effect of oxygen tension on the flash of *Noctiluca* (or of *Pennatula*) when stimulated, but this relation has not been determined.

the 30- to 300-mm range of oxygen pressure, and the intensity varied only 60%, the total amount of light increased only slightly with increasing oxygen pressure. The photogenic cell stimulation theory was therefore indicated, but other considerations led Snell to decide in favor of the tracheal valve theory.

One of these considerations was the demonstration that a flash *can* be obtained by sudden oxygen admission to a fire-fly kept at an oxygen tension below 4 mm Hg in which there is a faint continuous glow. The sudden increase in light intensity which appears under these conditions was called a "pseudoflash." It is considerably longer than a normal

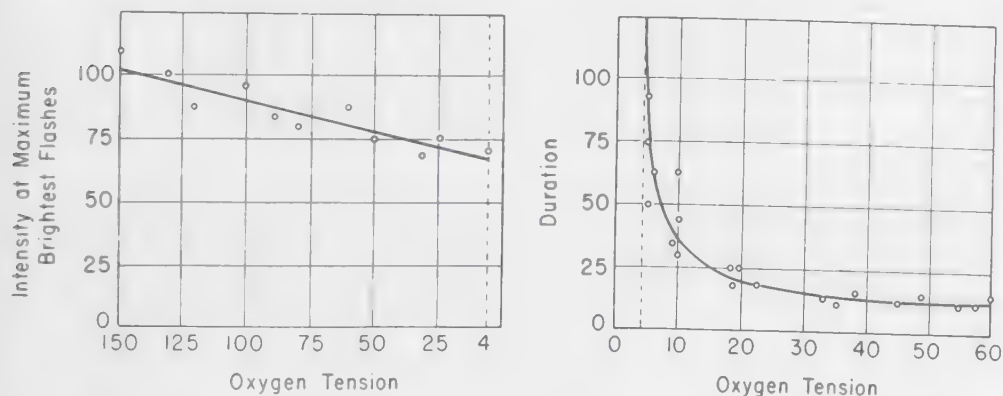


FIG. 138. Brightness of flash (left) and duration of flash (right) on vertical axis as a function of oxygen pressure in mm mercury on horizontal axis. After Snell.

flash, lasting about a second and is followed by darkness even though the fire-fly is kept in the atmosphere of higher oxygen. Records of pseudoflashes of *Photinus pyralis*, published by Alexander, are reproduced in Fig. 139. Note the difference in time scale, as compared with a normal flash.

The pseudoflash can be elicited either by increase of O_2 concentration or a sudden increase in air pressure, the important point being that the original oxygen level be below 4 mm so that the fire-fly shows a continuous glow and that the oxygen be suddenly raised to a point well above 4 mm. If the tracheal system is filled with water by immersing a fire-fly in water, evacuating, and then readmitting atmospheric air with the animal under water, no flash will be obtained on stimulation in air. There is also no glow at low oxygen pressures and no pseudoflash. These animals are not killed, as the water can be removed from the tracheal system and normal behavior and flashing regained. The experiment shows the importance of air filled tracheae for the flash.

Further analysis of the flashing mechanism has been carried out by Alexander (1943) in a study of high oxygen pressures. The bright glow which occurs when a fire-fly is placed in pure oxygen does not last indefinitely, but after a few seconds begins to dim. A similar bright glow with subsequent dimming also occurs when the air pressure is increased, in this case sometimes accompanied by an outburst of flashing, a reflex response of the animal to pressure changes. Except for this reflex flashing, Alexander found that pressure increase was equiva-

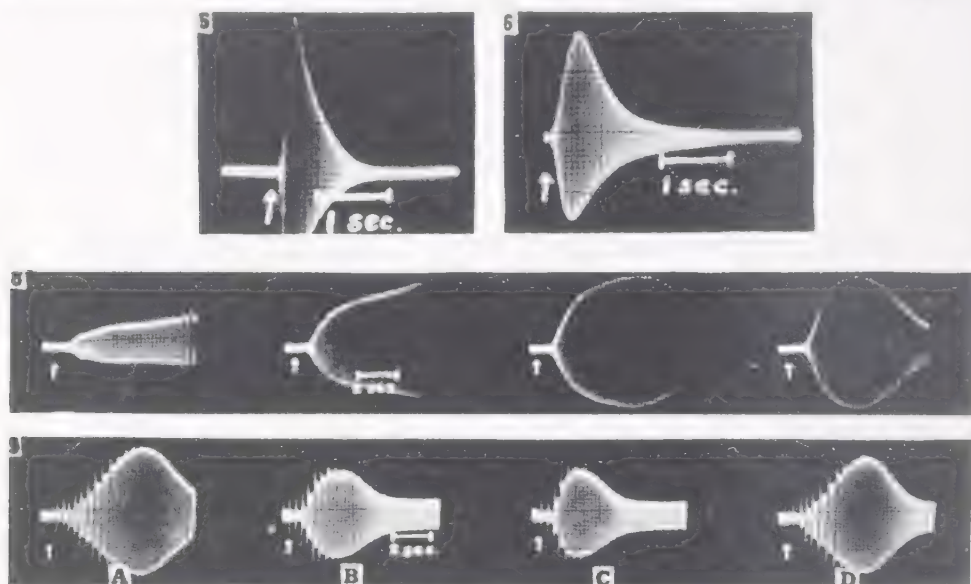


FIG. 139. Above, 5 and 6, pseudoflashes of *Photuris pennsylvanica* male (left) and *Photinus pyralis* male (right). Arrow indicates when pressure is applied. 8, effect of suddenly increasing air pressure (at arrow) about a fire-fly to 120 lb per sq in. for 10 seconds, then releasing the pressure for 20 seconds, then applying it again, etc. Note increase in rapidity of light emission and greater intensity but less duration with successive applications of pressure. 9, same, except time interval between pressure treatments is varied. Interval prior to A and D, 1 min; prior to B and C 20 seconds. Note difference in rate of appearance of light and also the flashing (light spikes) of the fire fly, during the period of light intensity increase but not during the decrease in light intensity, despite the fact that pressure was on from the arrow throughout the remainder of each record.

lent to oxygen tension increase, the steady glow beginning at 25 lb air pressure, the equivalent of about 400 mm O_2 found by Snell.

Alexander recorded the effect of sudden increases of air pressure to 120 lb maintained for ten seconds and then released. After an interval of 20 seconds the 120 lb was again applied for ten seconds and released. Altogether four applications of pressure were made and much information can be gained from study of these records, which are reproduced as Fig. 139. The first two records show only the initial

rise of light intensity, since the glow continued some time. It will be noted that the rise in light intensity occurs after a latent period and becomes more rapid in successive records while the total light becomes less. Figure 139 is another series taken after some preliminary pressure treatments. In these latter records spontaneous flashing, indicated by the spikes, is superposed on the glow due to increased oxygen. The time interval between C and D was one minute instead of twenty seconds. It will be observed that the build up of light in the D record is slower and the total light emitted is greater. It is also very apparent that flashing ceases at the peak of light intensity and does not occur during the decrease, even though the high oxygen pressure is being maintained.

Alexander has explained the latent period and increasing rate of build up in successive records as due to the time for diffusion of oxygen into the photogenic cells, independently of the flash mechanism. The more rapid rise in successive pressure applications merely reflects the oxygen already started on its diffusion pathway as a result of the previous pressure exposure.

The cessation of flashing at and after the peak of light intensity was explained in another way. When oxygen has reached the maximal concentration for luminescence, the rate of luciferin (or some other substance necessary for luminescence) formation or release becomes a limiting factor, and the light emitted decreases to a constant level reflecting that rate of formation. After this plateau intensity has been established, fluctuations in pressure between 50 and 100 lb have no effect on the intensity. Stimulation cannot produce a flash because there is no excess luciferin present. Since there is plenty of oxygen present, it is particularly significant that no flashing occurs at this time. Moreover, no flashes can be elicited by electrical stimulation during the plateau period, although the light intensity can be increased somewhat by electrical stimulation after a considerable latent period. Previous stimulation of the fire-fly before the application of pressure also increases the intensity of the high oxygen induced glow, as if pre-stimulation had released more luciferin for the photogenic reaction.

These slow changes in light intensity do not in any respect resemble a flash. The flash only appears when there are sufficient photogenic substances present and insufficient oxygen to maintain a maximum glow. Therefore Alexander has concluded that a nerve impulse to the tracheal end cells results in admission of a sudden burst of oxygen to the photogenic cells. The end cells behave like mechanical valves.

The author is inclined to agree that the tracheal end cell is the primary factor involved in a normal flash but is very dubious that it

serves as a mechanical valve. One objection is Snell's finding that the total light of a flash is not nearly proportional to oxygen tension. Control by the tracheal end cell would still be possible if stimulation released oxygen already stored in the end cell. Such oxygen secreting cells are present in the swim bladders of fish and are stimulated by nerve fibers running in the vagus. When more is known of this type of oxygen forming cell it may be possible to test the theory on the tracheal end cell of the fire-fly.

Microscopic Observation of Light Emission. Direct evidence in favor of the tracheal end cell as a controller of the flash is obtained by microscopic observation of the glowing lantern. All observers agree that a lantern in full brilliance reveals nothing but an intense homogeneous light. However, during submaximal luminescence, the light appears in localized areas. Spallanzani and many others noted tiny points of light which Schultze located in his newly described tracheal end cells. Bongardt and Lund also described the spread of light from minute flashing points until the whole organ was involved, and Lund identified the points of light as occurring "where the cytoplasm of the tracheal end cell and tracheole is applied to the photogenic cell." Frequently the light appears in the form of rings which Emery, Townsend, Lund, Alexander, and others have shown to be around the dark tracheal cylinders. According to most observers, the end cells are not luminescent.

Under low oxygen tension the rings of light become distinct points which Alexander also identified as the bases of the tracheoles, surrounded by their end cells, forming a stellate figure as they branch from the cylinder. The rapid repetitive flashing of these points can be observed under conditions when the nerve control is interfered with, due to vapors of ether or carbon disulfide, injection of various toxic substances, especially strychnine (Kastle and McDermott, 1910) and spider poison (Wood, 1939). Alexander noticed that "there is not the slightest indication of any local synchrony in the flashing of these tracheolar points. The tracheolar points around a given tracheal cylinder do not light up at once, *nor do the points of the tracheoles supplying a single photogenic cell light up at once.*"

This observation has particular significance. If nerves went to a photogenic cell, which is served by many end cells, the whole cell would light on stimulation, whereas it is only the region of the tracheal end cell which lights, and each end cell lights independently, strong evidence that nerves actually terminate on tracheal end cells and that under normal conditions they are responsible for initiating the flash when they fire together. In light organs without tracheal end cells there is observed only a uniform glow.

Action Potentials. The light organ of a fire-fly with its accompanying nerve might be compared to the classic nerve muscle preparation. During a flash there are undoubtedly chemical and thermal changes and Hasama (1939, 42) has reported electrical changes also. His first studies were made on *Luciola cruciata*, using non-polarizable Zn-ZnSO₄ electrodes and leading from luminous regions and non-luminous ventral body segments to a string galvanometer. The male of this species lights rhythmically 15 to 20 times a minute for a short time with 3 to 4 seconds between the pulses, while the female pulsates 20 to 30 times a minute with 2 to 3 seconds between the pulses. The "electroluminogram" showed the same rhythmic changes in potential, with the luminous region electronegative to the non-luminous. The potentials were intensified in oxygen and weakened or disappeared in ether and chloroform vapor. The action potentials of *Pyrocoelia rufa* also followed the rapid pulsations (in bursts of ten at a time) of 45 to 30 per second in the male and 65 to 105 per second in the female. The potentials and light were abolished in H₂ and CO₂ and increased in O₂. In ether and chloroform they first increased and then decreased and disappeared.

Hasama later studied the larvae of *Luciola cruciata* and *Pyrocoelia rufa* whose light is continuous and found that the action potential was monophasic and continuous but of various durations and broken by fluctuations and rest periods of different lengths. Potentials of the larva and adult of *Luciola lateralis* also followed the light emissions, so that the electroluminogram appears to be established on a firm foundation. Its origin in the organ, whether from nerves, tracheal end cells, or photogenic cells is unknown.

Thermal Changes during a Flash. This subject has been investigated by Coblentz (1912) in an extended series of experiments using thermopiles of various types and a galvanometer of high sensitivity. Some of the experiments appeared to indicate a cooling during active flashing as if the light-producing reaction were endothermic, but this effect could not be established with certainty and further work on fire flies, not too active and with a brilliant flash like *Photinus pallens* of Jamaica, are much needed. Coblentz concluded: "As a final summary of this perplexing series of experiments, it may be added that the temperature of the fire-fly is somewhat lower than the air, but it is not known whether this body temperature has a fixed value; that the luminous segments are hotter than the adjoining dark segments, this temperature difference being most marked on the ventral side of the insect, that it is uncertain whether this temperature difference is effected by the flash (whether the flash is accompanied by an endo-

thermic or exothermic reaction); and that, if radiant energy (especially the infra-red) is emitted by these insects, it is immeasurably small. No suggestions are offered as to the cause of this difference in temperature between the luminous and the non-luminous segments. Apparently there are slight discrepancies in the two sets of measurements of the effect of the flash upon this temperature difference; but a study of the data and of the functions of the two types of instruments used in these measurements does not indicate that this is a serious matter. To measure such a temperature difference is no small undertaking. To go one step farther and determine how this temperature is modified, as for example by the flash, is a different problem requiring further investigation."

Temperature and Luminescence. Almost every observer, including Hulme (1800), Macaire (1821), and Matteucci (1843), has noted the effect of temperature on one or another aspect of the luminescence of lampyrids. Fire-flies are fundamentally tropical and warm weather insects and in temperate climates do not appear if the temperature is too low. The light of an isolated mashed lantern can be seen at 0°C, and as the temperature rises, the light increases to an optimum and disappears at temperatures around 42–50°C, depending on the conditions. Lund (1911) has observed return of the light on cooling, especially in a *Photinus* species very similar to *P. maritimus*, and Kanda (1920) observed the same for the isolated lantern of *Luciola viticollis* whose light disappeared at 50°C.

It is an interesting fact, noted by Macaire (1821) and Lund (1911), and observed many times by the author (1924), that when fire fly lanterns are gradually heated, the color of the light takes on a definite reddish tinge just before it goes out. At 45°C the color is most red. The same change occurs with a lantern placed in alcohol, and McDermott (1911) noted the reddening just before the light disappeared in liquid air. When the animals were again warmed the first light was reddish, followed by the normal shade at higher temperatures.

The shift from yellow to reddish cannot be explained by shift of the eye from cone to rod vision but might be explained by the scattering of light from protein partly coagulated by heat or alcohol. The presence of protein particles would favor the transmission of red, just as at sunset the shorter wave lengths of the sun are scattered, but the red light penetrates the haze. At low temperatures minute ice crystals could act to scatter light and the effect would be reversible. The author (1944) was able to imitate the effect in a thin layer of egg albumen on a glass slide through which light with the same spectral distribution as the fire fly light was passed. As the albumen began to

coagulate on heating, the light changed from yellow to reddish. The experiment was quite convincing.

The best curve of temperature and light intensity has been obtained by McElroy and Strehler (1949), who used the partially purified photogenic substances from *Photinus pyralis*. The optimum is at 25°C. and the increase in light intensity with temperature indicates an activation energy (μ value) of 18,500 calories, about like that of luminous bacteria. The curve is reproduced as Fig. 140. Its resemblance to the

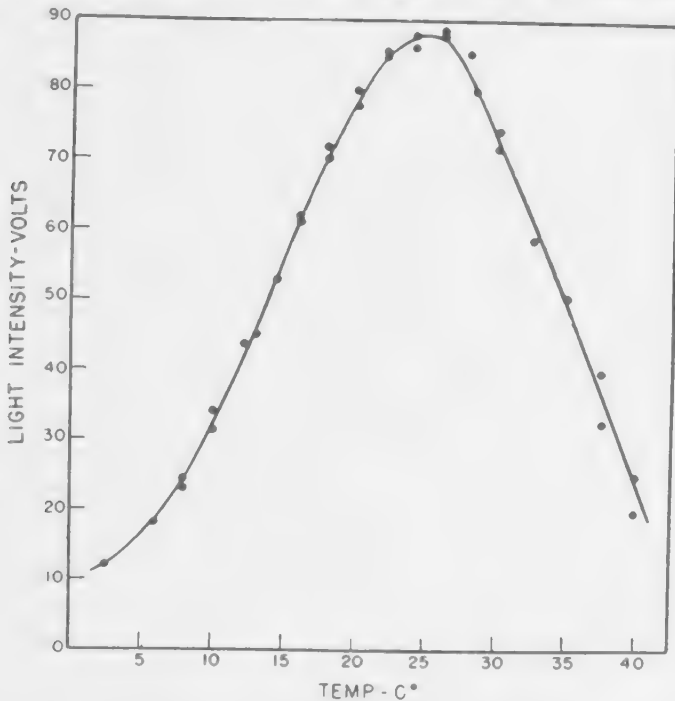


FIG. 140. Light intensity of the *Photinus pyralis* bioluminescent system *in vitro* (in arbitrary units) as a function of temperature. After McElroy and Strehler.

temperature-light intensity relation of luminous bacteria is very apparent.

The effect of temperature on the whole insect is more complex. Snyder (1920) studied the interval between successive flashes of fireflies (probably males of *Photinus pyralis*) and found it to vary considerably with temperature. The rate was from 8 per minute at 19°C to 16 per minutes at 29°C, giving a $Q_{10} = 2$. The rate was very constant at any one temperature, indicating that if a local group of fireflies became synchronous they would remain synchronous, provided the temperature did not change.

The observation indicates that the rhythmic flashing of the fire-fly is affected by temperature in a manner similar to other physiological rhythmic processes, like the heart beat. If the sexes detect each other

by the type of flashing, the rate will change with temperature, and hence the perceptive processes of the fire fly must also change correspondingly with temperature in order that recognition may occur at different temperatures. Buck (1937) has in fact observed that the interval between the flash of a male and the response of a female *Photinus pyralis* does decrease as the temperature rises, but nevertheless the male (at the same temperature) still recognizes the signal. His results are shown in Fig. 141, together with his observations on the interval between flashes of males at different temperatures.

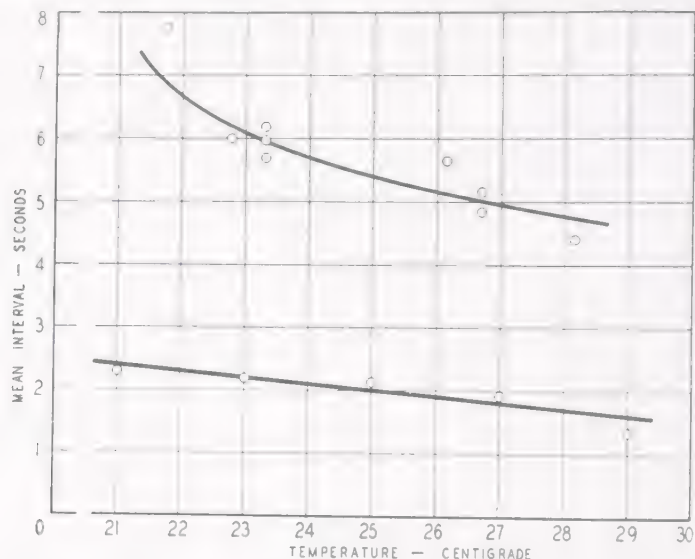


FIG. 141. Relation between temperature and flashing in the fire-fly. Upper curve, each point represents the average interval between successive flashes of males on one evening, plotted against the temperature which obtained on that evening; lower curve, each point represents the average length of the interval between the flashes of males, or of a flashlight, and the responses of females, over a range of 2°. From J. Buck, "The Signal System and Color Vision in *Photinus Pyralis*" in *Physiological Zoology*, published by the University of Chicago Press.

Effect of Pressure. Few really high pressure studies have been made on the fire fly. Dubois and Regnard (1884) exposed *Lampyrus noctiluca* in water in an opaque vessel to a hydrostatic pressure of 600 atmospheres for ten minutes and found on removing the glow worm that it was feebly luminous but non motile. It lived twenty two days but was evidently injured, and luminescence continued feeble. Another insect exposed to 600 atmospheres for ten minutes gave somewhat similar results. It was non luminous and could not be stimulated electrically to luminesce, but in three days had recovered and was luminous and vivacious. Dubois planned in later experiments to use a pressure vessel with a glass window to examine the light while under pressure but apparently never continued the work.

The effect of pressure on the isolated photogenic system of the fire

ily at different temperatures would be of great interest for comparison with the studies on bacteria and on Cypridina. It is hoped that knowledge in this field may be complete in the near future.

It is probable that in Dubois' experiments the gases in the tracheal system were forced into solution. With lower pressures in a gaseous medium, there is undoubtedly an effect of pressure to excite reflex flashing of whole fire-flies, as in the experiments of Alexander previously described. Ruedemann (1937) has observed fire-flies (*Photinus scintillans-marginellus*) set into rapid flashing by the explosion of cannon crackers, a possible effect of the pressure wave.¹⁶ Lund (1911) tested *Photuris pennsylvanica* in a glass vial connected with a rubber bulb that could be squeezed and observed that the flashing under pressure was brighter, not only with the whole fire-fly but also in the case of the continuous glow of excised lanterns and the isolated photogenic layer itself.

Effect of Light. Most fire-flies hide among leaves on the ground in the daytime and, if the temperature is high enough, come out only at twilight when the light intensity reaches a certain low value (Mast, 1911; Rau, 1932; Buck, 1937). This reaction does not necessarily mean that the ability to luminesce is affected by light, but the behavior pattern is affected. There is in fact a diurnal rhythm of lighting behavior in American fire-flies that has been studied by Allard (1931) and particularly by Buck (1937). Buck found in the laboratory that a change from bright light to a dim light induces flashing at any hour of the day, but if kept in continuous total darkness the frequency of flashing is relatively low. A change from darkness to dim light will cause flashing provided the fire-flies have been in darkness for 24, 48, 72, or 96 hours but not if they have been in darkness for 12, 36, 60 or 84 hours. This and other experiments indicate an inherent diurnal periodicity manifested by periods of flashing which recur at 24-hour intervals and persist for at least four days.

According to Pieron (1925), the glow-worm shows a rhythm lasting five days with irregularity after three days. Perkins (1931) found that the larva or female *Lampyris noctiluca* exposed to continuous light stopped glowing on the third day, whereas specimens kept in continual darkness or receiving diffuse light by day could be made to glow by electrical stimulation during night hours for fourteen days. Some have claimed that fire-flies require exposure to light¹⁷ for luminescence.

¹⁶ It has been observed by Pethen (1913) that the European glow-worm is sensitive to vibration and will light on tapping the ground as well as during a thunderstorm.

¹⁷ All experiments indicate that no bioluminescence is comparable to phosphorescence where the luminous substance must be previously exposed to sunlight or similar radiation.

but Peters (1841) and Matteucci (1847) observed the Italian Luciola to luminesce after eight days in darkness. There is no doubt a difference in behavior of various species whose details remain to be worked out.

The effect of bright light falling directly on the lantern is definitely inhibitory. In 1925 the author carried out experiments (unpublished) on males of *Photuris pennsylvanica* exposed to the parallel beam from a carbon arc which passed through 7.5 cm of water to absorb heat. Exposures of the lantern for two to five seconds were enough to diminish or prevent spontaneous or electrically induced flashes, but there was no effect of the bright light on the continuously glowing luminous matter of the lantern and no effect on flashing if only the eyes of the fire-fly were exposed to the light. The flash of *Photuris* females appeared to be less easily inhibited by light.

When a series of flashes is inhibited and disappears as a result of exposure of the lantern to bright light and the light is then turned off, at first very faint flashes appear which gradually increase in intensity until they are again normal. The stimuli for the flash are apparently sent to the lantern, but the light inhibits the flashing mechanism rather than the photogenic process. The steady brilliant glow of the lantern induced by adrenaline injection is not inhibited by light in an atmosphere of oxygen.

Effect of Various Substances. Research dealing with the effect of various compounds on the flash and on the luminous matter of the lantern is in a most unsatisfactory state because of the complex function that is being observed. When a whole animal is studied, it is often impossible to distinguish between effect on the air supply through the tracheal system or effects on the nervous system, or on the excitation process, or the photogenic system itself.

Every conceivable type of compound has been tested on the fire-fly, but in many cases actual concentrations are not known, or proper controls were not kept, or the observation may have been made only once. The fire fly is notoriously variable in behavior, and interpretation of results of exposure to a gaseous phase or injection of solutions into them requires highly critical judgment. An immense volume of data will be found in various papers. The earlier work has been reviewed by Kastle and McDermott (1910), and the reader is referred to this paper for details.

A few observations have been made on the isolated luciferin-luciferase-adenosine triphosphate system of the fire fly by McElroy and Strehler (1949). Certain oxidative inhibitors are of special inter-

est. Sodium azide, sodium fluoride, urethane and 2-methyl-1, 4-naphthoquinone in proper concentration all inhibit, but 0.001*m* NaCN has no effect, a general finding for cyanide in bioluminescence reactions. Kastle and McDermott (1910) found no inhibition of the light of excised lanterns, and the author (1917) observed no effect of cyanide on crude glowing extracts of the Japanese fire-fly, *Luciola viticollis*.

EPINEPHRINE (ADRENALINE). The injection of many different substances into a fire-fly will result in a bright constant glow. Kastle and McDermott (1910) wrote: "Without entering into the details of all of our own experiments, it may be said that strychnine, adrenalin, hydroxylamine sulphate, hydrazine sulphate, the nitrates of sodium, potassium and barium, sodium hydroxide, sodium acetate, sodium bicarbonate, disodium phosphate, sodium fluoride, and sodium sulphite, all acted as powerful stimuli to light production, whereas pure water, sodium chloride, sodium bromide, sodium nitrate, sodium benzoate, potassium nitrate, potassium iodide, barium chloride, etc., showed but little or no effect."

Of these compounds, adrenaline has aroused most interest because it is a hormone, has a definite action on vertebrate arterioles and is known to excite luminescence of photophores when injected into fish. Also because histological examination of adrenaline-injected fire-flies by Creighton (1926) revealed that the tracheoles within the photogenic cells were dilated, as compared with tracheoles of fire-flies injected with physiological salt solution, which does not induce the constant glow.

Creighton interpreted the adrenaline effect to be on the valve mechanism described by Dahlgren (1917). The contractile fibers in the tracheal end cells were caused to contract and thus distended the elastic ring, allowing more air under pressure in the tracheal system to be forced into the photogenic cells. The expanded tracheoles resulted from opening of the valve.

Emerson and Emerson (1941) have confirmed the epinephrine effect and shown that it is not due to impurities in the commercial adrenaline employed by Creighton. Other sympathicomimetic amines have a similar effect. Phenethylamine, phenethanolamine, neosynephrine, tyramine, and hordenine gave a bright glow, while benzedrine, propadrine, and *L*-ephedrine glows were of moderate intensity. They concluded that "The action of epinephrine is not mediated through direct chemical stimulation of the luminescence reaction. Consideration of relative potencies of its congeners in evoking constant luminescence in fire-flies suggests that the site of action is at the tracheal end cells and that no nicotine-like effect is involved."

Seifter (1945) has reported that amphetamine in 0.5 to 2% solution, when painted on the abdomen of a fire-fly, causes the normally intermittent glow to become continuous for hours at a time and the more concentrated solutions to result in longer action.

ALKALOIDS AND SCINTILLATION. Another action of various substances on the fire-fly lantern is that of scintillation. Kastle and McDermott (1910) wrote: "Strychnine, hydroxylamine sulphate, and the nitrates were especially remarkable in their effect. . . . When a drop of a solution of strychnine sulphate was injected into a live, active fire-fly, it was observed that invariably the insect gave one or two strong flashes of light, after which the luminous organ became quiescent and the insect apparently dead. In a short while, however, the entire luminous organ began glowing, and a great many bright scintillations or bright points of light followed one another in rapid succession over its entire area." Wood (1939) observed this "spinthariscopes" activity in a fire-fly poisoned by a spider, and Alexander (1943) after various treatments. The effect is undoubtedly on the central nervous system and comparable to the effect of strychnine in stimulating a succession of discharges from motor cells of the cord of a vertebrate.

ANESTHETICS. Kastle and McDermott (1910) found that in ether vapor whole fire-flies (*Photinus pyralis*) and also excised lanterns show a few faint flashes at first but then burst into a steady glow which dies out after some time. The isolated organ in methyl and ethyl alcohol vapor glowed brightly and then died out after a final series of "brilliant luminous pulsations." According to Gerretsen (1922), who confirmed the earlier work of Verworn (1892), *Luciola italica* and *L. vittata* exhibit three stages in the action of CHCl_3 vapor: (1) reversible inhibition of flashing, sometimes accompanied by a faint glow and cessation of muscular activity; (2) an irreversible bright glow; (3) irreversible extinction of light. There is no doubt that the nervous system is anesthetized in stage 1, but the effect in stage 2 might be due to direct stimulation of photogenic cells or relaxation of some tracheal end cell mechanism of control. In stage 3 a damage to the photogenic system is indicated. The author (1917) found that *Luciola vitticollis* extracts rapidly lost their luminescence when saturated with ether or chloroform, and Buck (1948) has observed that lanterns of *Photuris pennsylvanica* whose light has disappeared in ether vapor will not luminesce when exposed to air and teased apart.

The most careful experiments on anesthetics and luminescence are those of Emerson (1935), who also used *P. pennsylvanica* and known mixtures of ether vapor and air. The fire-flies were first injected with adrenaline which causes a bright constant glow for over four

hours. Low ether concentrations abolished motility without affecting the glow, while higher and higher concentrations put out the glow in less and less time. The highest concentrations were lethal to the animal. Emerson concluded that the dehydrogenase system (luciferase) involved in luminescence was affected by ether in relatively high concentrations and that the constant glow was due to dilation of tracheoles. This conclusion may be questioned, since Buck (1948) has found that the glow of *P. pennsylvanica* larvae, which lack tracheal end cells, behaves toward ether like the adults. The early stages of glow induced by ether are reversible, according to Emerson and Buck (1946), a point missed by Verworn and Gerretsen. Studies of the effect of anesthetics on the isolated photogenic system of the fire-fly are needed to aid in interpretation of the observations.

Biochemistry. The earliest notions on luminescence of lampyrids attributed the light to phosphorus or to formation of some organic compound of phosphorus akin to phosphine. The fact that the fire-fly lantern could be dried and would again luminesce on moistening (Caradoti in 1798; Carus, 1829, 64) and the necessity for oxygen were early discoveries. The presence of a photogen of some kind was obvious, and the demonstration of luciferin and luciferase by Dubois in *Pyrophorus* and *Pholas* laid the ground work for experiments of the twentieth century.

Early attempts (McDermott 1911, 15; Harvey 1914, 15) to extract luminous materials from the fire-fly were not very successful. It was possible to show that fat solvents like alcohol, ether, chloroform, toluol, or acetone did not dissolve anything from the dried powdered lanterns that noticeably diminished the light emission when water was added to the previously extracted tissue. Attempts to extract with oxygen-free aqueous solvents also gave negative results. The dried lanterns lost their ability to luminesce after some months, even if preserved with desiccating agents, and fire-fly material did not at that time appear favorable for biochemical work.

Luciferin and Luciferase. The classical luciferin-luciferase reaction in lampyrid fire-flies was announced by the author in 1916, but the resultant light, compared with that of Cypridina, was weak and short lasting. In this early work on luciferin and luciferase it was observed that not only would the heated (and cooled) extract of lanterns produce light when mixed with a cold water extract of the lanterns, but that extracts¹⁸ of non-luminous parts of the fire-fly and extracts of non-

¹⁸ These substances in tissue extracts were at one time (Harvey, 1916) referred to as photophelms or light assistants (from the Greek *phos*, light, and *phelin*, to assist) and were compared to coenzymes of yeast, while luciferase was compared to

luminous insects also produced light with "fire-fly luciferase." However, no light appeared from the converse mixture — "fire fly luciferin" and extracts of non-luminous fire-fly tissues or non-luminous insects.

Adenosine Triphosphate. Explanation of these effects is to be found in recent experiments by McElroy (1947) which have indicated that emission of light by the fire-fly involves more than the luciferin-luciferase system. If the lanterns of a fire-fly are extracted with cold water and the extract is allowed to stand until the light disappears (fire-fly luciferase solution) the addition of adenosine triphosphate (ATP) will result in a bright, long-continued luminescence, a much more striking luminescence than that observed when a hot water extract (allowed to cool) of fire-fly lanterns (fire-fly luciferin) is added. It appears that in fire-fly luminescence the high-energy phosphate bond is necessary, by means of preliminary transphosphorylating dark reactions, to build up the total energy available for excitation to around 50 kilocalories, the quantum energy equivalent to the emission maximum (in the region $\approx 0.56 \mu$) characteristic of *Photinus pyralis* light.

That the action of ATP, which is universally distributed in nature, accounts for the early observations (Harvey, 1916) on the activity of non-luminous extracts in producing light when added to "fire-fly luciferase" is confirmed by the fact that these extracts caused luminescence if they were fresh but not after they had stood for an hour. However, if first boiled, they retained for a long time their ability to cause luminescence when added to fire-fly "luciferase." The probable explanation is that both ATP and adenosine triphosphatase (ATP-ase) were present in the extracts and that ATP-ase destroyed the ATP, unless the extract had been boiled. Thus the light-producing effect of an extract of non-luminous tissue was probably due to the ATP it contained rather than the presence of luciferin, as had been supposed.

The effect of ATP in prolonging the luminescence of fire fly lantern extracts offers another interpretation of a cross experiment of the author (1917) in which "luciferin" from *Photuris pennsylvanica* with a yellow light was mixed with "luciferase" of a *Photinus* sp. having an orange light. The reverse cross, *Photuris* luciferase and *Photinus* luciferin, was also tried. It was found that the color of the light was characteristic of the animal supplying the luciferase, and the original interpretation was that luciferase molecules were the light emitters. It is probable that the luciferin solution merely contained ATP, and the

the enzyme proper and regarded as the light emitter. However, work with the ostracod crustacean, Cypridina, quickly led to the abandonment of this view (Harvey, 1918). It is possible that luciferin may still be regarded as the coenzyme of luciferase.

color of the light was determined by the luciferin-luciferase system of the respective fire-flies.

Purification and Properties of Luciferin. McElroy and Strehler (1949) have been able partially to purify and separate the fire-fly luciferin-luciferase system of *Photinus pyralis* by $(\text{NH}_4)_2\text{SO}_4$ precipitation. The precipitate will redissolve and can be used for quantitative study of the light emission. No light appears when the precipitate is dissolved in water, but whenever ATP is added, luminescence occurs. The intensity is proportional to the ATP added, up to a limiting value. The luciferin has not been exhausted, and presumably its ability to emit is reestablished by transfer of energy from the high-energy phosphate groups of the ATP molecule.

It was also found that activity lost during purification of the system could be restored by adding one of a number of inorganic ions. Mg, Mn, and Co were most effective, particularly Mg, and it is significant that these are known to be necessary for transphosphorylase systems. An analysis of the luminous organs of *Luciola cruciata* by Kawanaka and Matsubara (1941) has indicated the presence of Fe, Zn, Ca, Li, and Na.

The isolated luciferin-luciferase system gave the same emission spectrum as the fire-fly itself with a maximum at 565 $m\mu$. Its pH optimum was 7.5 in sodium phosphate buffers. Potassium phosphate buffers depressed the luminescence somewhat. The temperature optimum was 25°C and the activation energy 18,500 calories. As previously mentioned, cyanide and azide had little effect on the system while 2-methyl-1, 4-naphthoquinone was inhibitory. A recent (McElroy and Coulombre, 1951) improved purification technique has concentrated the light emitting system one hundred times.

During the ammonium sulfate purification studies on the fire-fly luciferin-luciferase it was noticed that a dialyzable factor present only in the light organs became limiting for luminescence. This factor has been separated from lanterns of *Photinus pyralis* by a number of methods and turns out to be fire-fly luciferin (Strehler and McElroy, 1949). The crude concentrated dialyzate of fire-fly lanterns can be partitioned with butyl alcohol which takes up the luciferin, or chromatographic methods can be used. The most effective adsorbants are mixtures of CaCO_3 and celite, and MgO and celite with butyl alcohol as the developing solvent. Paper chromatography is likewise effective. The luciferin can also be precipitated by Ag_2SO_4 from water solution at 0° and the silver salt decomposed with H_2S .

The luciferin obtained by these methods is soluble in water, methyl, ethyl, propyl, butyl, isobutyl, amyl and isoamyl alcohol, acetone and

ethyl acetate, but is relatively insoluble in benzene, petroleum ether, ethyl ether, and carbon tetrachloride. The partition with butyl alcohol is facilitated by acidification. The purified luciferin differs from *Cypridina* luciferin in being stable to oxidation, no loss of activity occurring when aerated for eighteen hours at 28° C or in boiling for thirty minutes, but in crude extracts of fire-fly lanterns, it is destroyed on heating to 80° C for two minutes. Drying at room temperatures, acids and alkalis and oxidizing agents destroy it. Absorption spectra at different pH values are shown in Fig. 142. The luciferin gave a

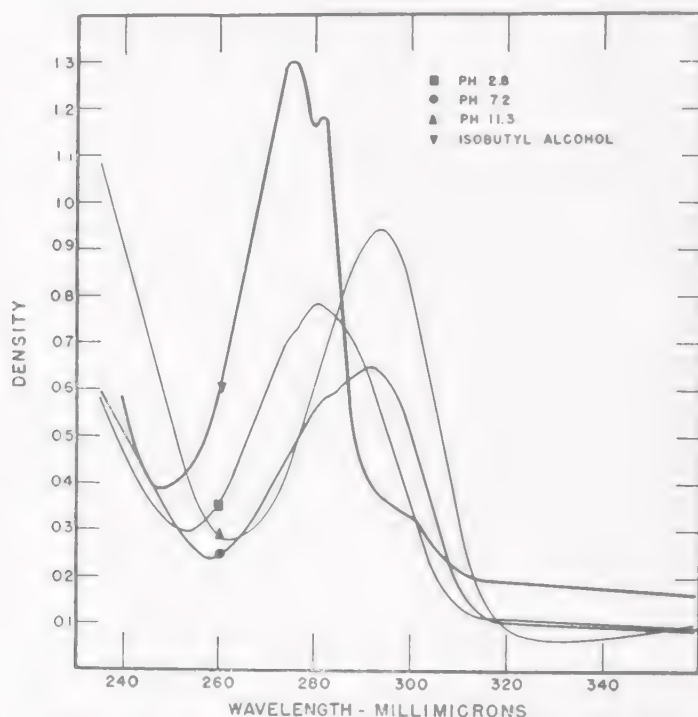


FIG. 142. Absorption spectrum of *Photinus pyralis* luciferin in isobutyl alcohol and in phosphate buffer at various pH values. After Strehler and McElroy.

negative ninhydrin test and a negative color reaction for paranaphthoquinones by the acetoacetic ester method of Ashley and Raistrick.

The luciferin has a blue fluorescence in the acid range; in the alkaline range there is a yellow green fluorescence, similar to the bioluminescence. Both *Photinus pyralis* and *Photuris pennsylvanica* contain the luciferin, present only in luminous organs. *Photinus pyralis* also contains a number of other fluorescent compounds through-

¹⁰ Dr. McElroy has informed me that he has been able to keep a water extract of lanterns dried over CaCl_2 for two years at -18°C and found it to luminesce after thawing, when ATP was added.

out the body that have been known for a long time under the name of luciferesceine.

Luciferase. The purification and properties of fire-fly luciferase have not yet reached a point to warrant discussion.

The Photogenic System in Different Fire-Flies. It is apparent that water, oxygen, luciferase, luciferin, ATP, and Mg or Mn salts are all essential for luminescence in the fire-fly. Lack of any one of the six factors will prevent luminescence. When water extracts of the light organs of at least five North American species (*Photinus pyralis*, *P. scintillans*, *Photuris pennsylvanica*, and two unidentified) fire-flies are made, the ATP is the first factor to be used up or to disappear. Hence adding ATP to the extract will restore the luminescence. Recently McElroy and Harvey (1950) have investigated a number of Jamaican fire-flies and found some (*Photinus melanotis* and *Diphotus montanus*) in which the luciferin also disappears. Adding ATP alone to an extract will not restore the light, but the addition of both ATP and luciferin prepared from *P. pyralis*, does result in luminescence. Some North American and Jamaican larval forms (glow-worms) belong in this category, also. Finally, in one species, *Photuris jamaicensis*, the extract was found not to respond by luminescence when both ATP and *P. pyralis* luciferin were added. This result is due to disappearance of luciferase, as the luminescence of *P. jamaicensis* extracts returned when dark extracts of lanterns of *Diphotus montanus*, which lack ATP and luciferin but contain luciferase, were added. These results indicate that the luciferin of one fire-fly will react with the luciferase of another species, provided all the other necessary constituents are also present. It also emphasizes the complexity of the luminescent reactions and the lability of some of the factors.

Luciferesceine. The existence of fluorescent substances in insects and in fact throughout the living world is widespread. Since the development by Wood in 1919 of filters to pass ultraviolet without the visible, innumerable papers have described the fluorescence of this or that living tissue (see Harvey, 1926).

Dubois (1886) appears to have been the first to call attention to a blue fluorescent material in *Pyrophorus*, pyrophorine, which he thought aided the beetle in light production by absorbing invisible rays and converting them into visible ones. He also noted the presence of this compound in *Luciola italica* and later (1911) adopted the name luciferesceine for the fluorescent compound.

That the fluorescence cannot take part in light emission of fire-flies was shown by Coblentz (1909, 12), who photographed the emission spectrum of *Photinus pyralis* and the fluorescence spectrum of the

luciferesceine from this fire-fly. These spectra do not overlap and no wave lengths of fluorescence emission (from 388 to 492 $m\mu$) appear in the fire-fly light (from 501 to 668 $m\mu$). The fluorescence emission maximum was at about 410 $m\mu$, the bioluminescence emission at about 565 $m\mu$. Coblenz found abundant luciferesceine in *Photinus pyralis*, *P. consanguineus*, *P. scintillans*, and *Ellychnia corrusca* (non-luminous in late adult stages), but very little in *Photuris pennsylvanica*.

MacDermott (1911) studied the properties of luciferesceine obtained by alcoholic extraction of the sticky defensive secretion exuded by fire-flies when irritated, but was unable to crystallize it. The material was soluble in water, ethanol, amyl alcohol, and glycol, slightly soluble in ether and insoluble in benzene, chloroform, acetone, and carbon tetrachloride. He regarded the fluorescent material as an incidental compound found in many species of Lampyridae and connected with the defensive function of the insects. Luciferesceine, in fact, is quite abundant in wings and other non-luminous parts.

The investigations of Strehler and McElroy (1949) have indicated that in addition to fire-fly luciferin a variety of green, blue, and violet fluorescent compounds occur in both luminous and non-luminous parts of *Photinus pyralis* (not in *Photuris pennsylvanica*) which have probably been grouped together as luciferesceine by previous writers.

Strehler (1950) has undertaken the isolation of "luciferesceine" from *Photinus pyralis*. The insects were dried *in vacuo*, pulverized, extracted with ethyl ether to remove fat and then with ethyl acetate. On removing the ethyl acetate *in vacuo*, the residue dissolved in water with strong fluorescence, and the mixture of substances was separated in a "chromotrain" with a water-butyl alcohol system. Certain fractions contained a substance which would dissolve in ethyl acetate and could be precipitated with hexane. Fine white platelets and rosette crystals separated from a methyl alcohol solution of the precipitate at -20°C . From 30,000 fire-flies (without light organs) there was obtained 35 mg of the material.

This compound had the empirical formula, $\text{C}_{11}\text{H}_{13-14}\text{O}_4\text{N}_2$, and many properties of a pteridine derivative, amine substituted, and with a polyhydroxy side chain. Its lowest molecular weight would be 282. The compound is very soluble in methanol and ethanol, less so in water, propanol, and butanol and nearly insoluble in ether and hexane. The fluorescence emission spectrum, shown in Fig. 143, has a peak at 410 $m\mu$ at pH 8.1 and at 405 $m\mu$ at pH 0.2. In water solution the fluorescence is deep blue-violet. The absorption spectrum peaks are at 257, 283, and 355 $m\mu$ at pH 11 and at 291.3 and 346 $m\mu$ at pH 2. One pK_a is 8.1 to 8.2. Strehler believes that this compound may be closely allied to

and possibly a precursor or a degradation product of fire-fly luciferin, whose fluorescence maxima are at $535\text{ m}\mu$ at pH 11.8 and at 535 and $410\text{ m}\mu$ at pH 7.63. The absorption spectrum of luciferin shows a peak around $290\text{ m}\mu$ (see Strehler and McElroy, 1949). The bioluminescence emission spectrum obtained by McElroy and Rainwater (1948) for fire-fly (*Photinus pyralis*) extracts has a maximum at $554\text{ m}\mu$. Both spectra are shown in Fig. 144.

Flavin. As in many luminous animals, another type of fluorescence is very evident in the light organ of the fire-fly. Like other luminous

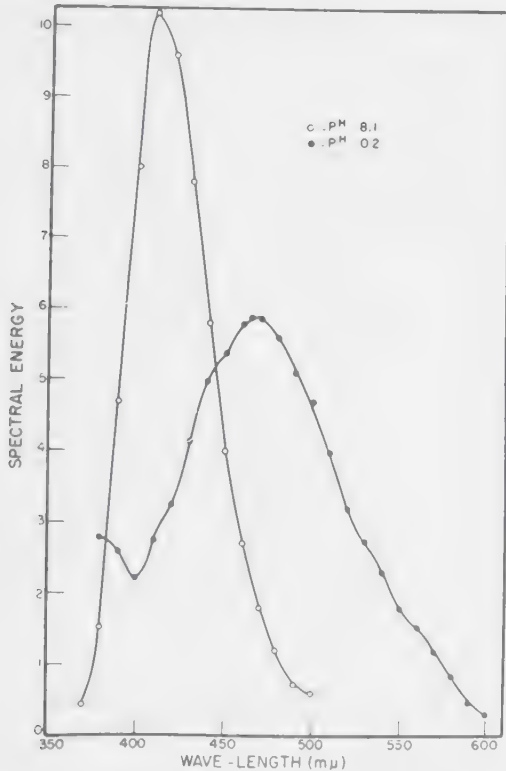


FIG. 143. Fluorescence emission spectra of luciferesceine from *Photinus pyralis* at two different pH values, 8.1 and 0.2. After Strehler.

species the light organ of an intact fire-fly appears to be luminescent when examined in ultraviolet light. The effect is so striking that only an opaque screen before the ultraviolet lamp will convince the observer that he is not examining a bioluminescence. The color of the light is also approximately like that of the bioluminescence, but a microscopic examination reveals a difference. In an unpublished investigation in 1926, the author made cross sections of a fresh abdominal segment containing the lantern and found that in ultraviolet light a pale yellow fluorescence came from both reflector and photogenic layer. The line

of demarcation between the two could not be distinguished. However, when the bioluminescence appeared, it was confined to the photogenic layer alone and might be yellow in *Photuris pennsylvanica* but definitely orange in a species of *Photinus*. It is very possible that the fluorescence comes from a flavin compound. The remainder of the abdominal tissues fluoresce blue from the "luciferesceine" which they contain. The larval light organ of glow-worms also shows pale yellow fluorescence in ultraviolet light and the fat body a violet fluorescence.

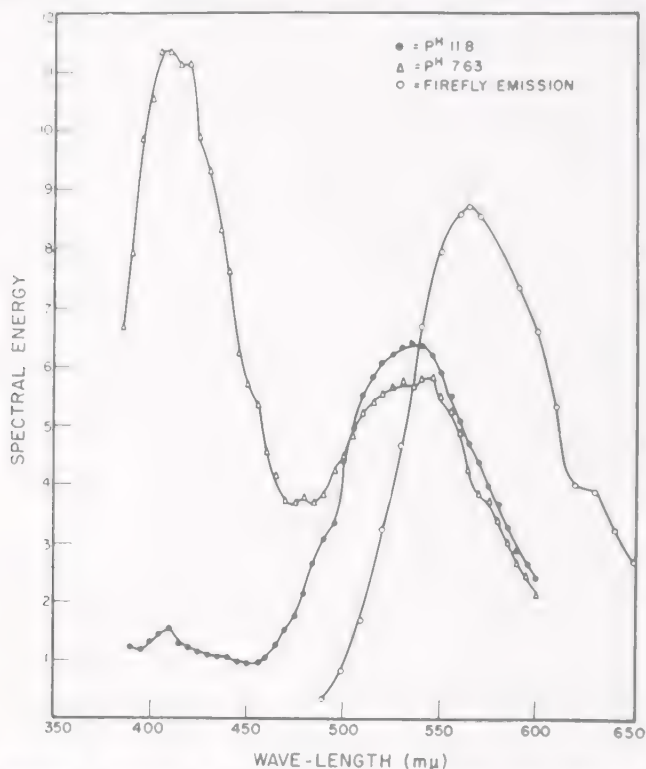


FIG. 144. Spectral emission of *Photinus pyralis* bioluminescent system *in vitro* (open circles), after McElroy and Rainwater, and fluorescence emission spectra of the *Photinus pyralis* luciferin at two pH values, 11.8 (solid circles) and 7.63 (triangles), after Strehler.

As flavins are of widespread occurrence in nature, it is not surprising to find them in fire-flies. During the examination of frozen sections of the luminous organs of the female *Lampyrus* in filtered ultraviolet light, Brooks (1940) noticed mostly a faint bluish fluorescence but a yellow fluorescence in some regions. On treatment with acetic acid which dissociates flavin-protein compounds, the yellow fluorescence became very marked and an assay indicated that 18.5 gamma of flavin per gram of fresh material was present.

Ball and Ramsdell (1944) have likewise analyzed American fire

flies from the neighborhood of Baltimore and find a flavin-adenine dinucleotide content of 36 to 70 gamma per gram of dry lantern material, whereas the rest of the non-luminous fire-fly tissue contained only 15% of the lantern value. As the lantern contains about 75% of water, this indicates a high content of flavin-adenine dinucleotide, although it is only one-fourth to one-half of dry mammalian liver content, one of the tissues richest in this flavin compound. The high flavin content of the lantern may have special significance, especially in view of the studies of Komarek, Wenig, and Backovsky, who attribute the luminescence of the earthworm, *Eisenia submontana* directly to flavin.

Lampyrine. Another fluorescent pigment, lampyrine, has recently been isolated by Metcalf (1943) from the Lampyridae. It is responsible for the rose coloration of the pronotal disc of many species (listed by Metcalf), and also occurs in the red adipose tissue and male gonads of *Photinus marginellus*. The rose-red fluorescence of this pigment in ultraviolet light is easily recognized in dried specimens.

Lampyrine occurs as spherites, 0.7 to 3 μ in diameter, insoluble in water, methanol, ethanol, ether, chloroform, acetone, carbon disulfide, formaldehyde, pyridine, and acetic acid, but soluble in 5% HCl. In this solvent the fluorescence spectrum extends from 655 to 563 $m\mu$ with maxima at 635 $m\mu$ and 600 $m\mu$. When a warm solution of the pigment in 5% HCl is cooled, pink fluorescent doubly refracting crystals, melting at 315°C will separate out. Lampyrine properties differ from other red fluorescent pigments like the chlorophylls, porphyrins, and polyhydroxyanthraquinones, and Metcalf thought it might be related to the pterins, widely distributed among the Lepidoptera.

It is attractive to speculate that lampyrine might possibly be involved in determining the color of the light of different fire-flies which vary from greenish yellow to orange. The orange color might be due to fluorescence of lampyrine excited by fire-fly light. Experiments to test this possibility have not yet been carried out, and at present the pigment is not known to be concerned with luminescence.

Enzymes. Very little is known of the enzyme content of fire-fly lanterns, apart from the fact that a compound which may be designated luciferase is present.

When Loew (1901) discovered catalase, the enzyme which decomposes H_2O_2 , he determined that there was no more of this enzyme in the luminous tissues of fire-flies than in other regions. Kastle and McDermott (1910) also mention the presence of catalase and peroxidase, since a fresh extract of the lanterns would cause bluing of gum guaiac only if H_2O_2 was added.

Burge (1916), as a measure of oxidative processes, determined the

amount of oxygen liberated in ten minutes from 30 cc of hydrogen peroxide (probably 3%) by 30 mg of the insect ground in a mortar with sand. He concluded "that the oxidative processes of luminous insects such as the fire-fly, are more intense than of non-luminous insects, such as the moth, butterfly, etc; that the oxidative processes in the luminous parts of the fire-fly are probably more intense than in the non-luminous part." Tsiro (1937) has also discussed the significance of catalase, but no definite activity in luminescence has been proved.

Physical Properties of the Light. The brightness of the fire-fly and the wide distribution of various species have favored study of the physical properties of the light, which is not polarized. Intensity, spectrum, and penetrating radiation have been very thoroughly investigated.

Intensity. Light intensity is measured in terms of candles and Prochnow (1905), using a bunsen grease photometer, determined that 6,400 brightly lighting females of *Lampyrus noctiluca* were the equivalent of one normal candle.²⁰ American fire-flies are somewhat brighter. Coblentz (1912) has estimated for the flash of *Photinus pyralis* around $\frac{1}{400}$ to $\frac{1}{50}$ candle, while the continuous steady glow sometimes observed in this insect was of the order of 1/50,000 candle.

The brightness of the fire-fly light is of more interest than the intensity, and Ives and Jordan (1913) found 0.0046 candle per square centimeter or 14.4 millilamberts for the intrinsic brilliancy of an American glow-worm. Ives (1922) calculated that an area 2 meters in diameter covered with luminescent material of 14 millilamberts brightness on the ceiling of a room would be sufficient for working on a table 2 meters below.

Spectrum. Spectral studies have particularly interested investigators. Murray (1826) was probably the first to examine *Lampyrus noctiluca* light with a prism and to report that it "seems monochromatic and incapable of further decomposition," but Lehmann (1862) and Schnauss (1862) found that there were present red, yellow, and green components. Schnauss also noted that *Lampyrus* light would affect a photographic plate. *Pyrophorus* light was shown to give a continuous band without lines and without infrared or ultraviolet rays by Pasteur (1864). Young (1870), Secchi (1872), Severn (1881), Conroy (1882), Spiller (1882), Ives and Coblentz (1909), McDermott (1910), Ives (1910), Coblentz (1912), Ramdas and Venkiteshwaren (1931), Yosida, Nakamura, and Okada (1935), Buck (1937, 41), and Grinfeld (1944) have established without question that the spectra of lampyrids are short bands lying in various regions, with different maxima. Buck

²⁰Geipel (1944) stated that the male *Lampyrus splendidula* had a "Beleuchtungsstärke" of 0.00008 "Meterkerzen."

has given a table of the limits of spectra previously found and added spectrometric data from spectral photographs of twelve Jamaican species of lampyridae. There was no essential spectral difference in the light of males and females.

The spectral energy curve is a more valuable determination than a photograph of the spectrum. The classical work in this field has been carried out by Ives and Coblenz, and Coblenz, who have determined the spectral energy curve and maximum emission of four species of fire-fly. The maximum emissions are as follows: *Photuris pennsylvanica*

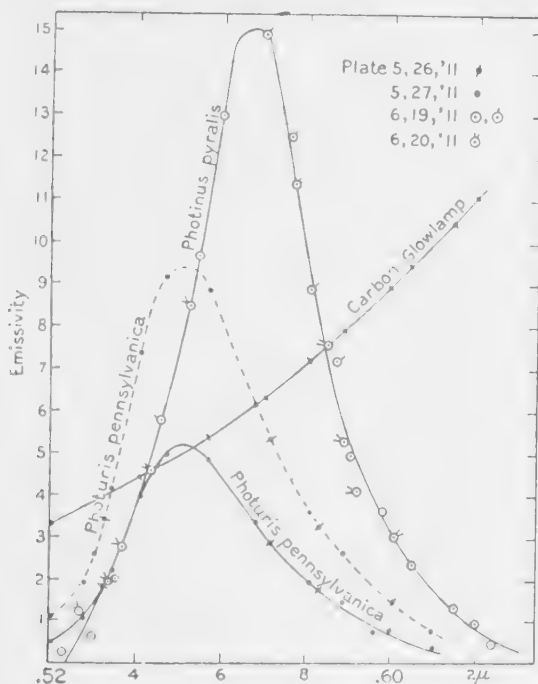


FIG. 145. Spectral energy curves of various fire-flies and a carbon incandescent lamp. Wave length in micra on horizontal. After Coblenz.

vanica both larva (glow-worm) and adult, at 552 $m\mu$; *Photinus pyralis* at 567 $m\mu$; *P. consanguineus* at 578 $m\mu$; *P. scintillans* at 578 to 580 $m\mu$. The maximum emission of *Pyrophorus* was set at 538 to 540 $m\mu$. Some curves are shown in Fig. 145, and it will be noted that they are quite symmetrical.

Since partially purified extracts of fire-fly lanterns with a long-lasting luminescence have become available, McElroy and Rainwater (1948) have determined the spectral energy emission curve for such extracts of *Photinus pyralis*. The result is reproduced as Fig. 144. It will be noted that the maximum is at 553 $m\mu$ and a "hump" appears in the long wave region at about 650 $m\mu$. There was no reddening of

the light when the temperature of the extract was raised, as is the case when the lantern of the living insect is heated.

No ultraviolet or infrared rays were present in the photographs of the spectra, and Ives (1910) made a particular effort to detect ultraviolet by fluorescence and infrared by its effect in quenching the phosphorescence of Sidot blende. He came to the conclusion that, except for the short band in the visible, no radiation is emitted between 200 m μ and 1.5 μ . Coblentz (1912) has measured the infrared absorption of the cuticle of the fire-fly and obtained the curve shown in Fig. 146. Okada, Kōisi, and Yasuda (1937) state that the chitin of the lantern is transparent to ultraviolet.

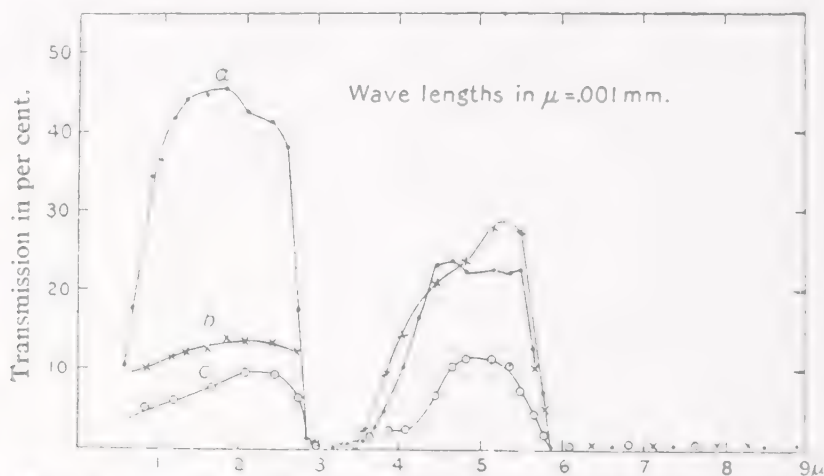


FIG. 146. Transmissivity of the chitinous cuticle of fire-flies to infrared radiation. Wave length in micra on horizontal. a, *Pyrophorus noctilucus*, abdomen; b, *Pyrophorus noctilucus* prothoracic luminous organ; c, *Photinus pyralis* lantern. After Coblentz.

As both Ives and Coblentz have pointed out, practically all the radiation is in the visible, and hence the radiational efficiency (visible light times visual sensibility divided by total radiated energy) is of the order of 90%, whereas in a carbon glow-lamp the value is 0.43%. McDermott and Ives (1914), Karrer (1918), and Ives (1915, 22) have discussed these high efficiencies in relation to engineering problems, hoping that a knowledge of "living light" might help solve the extraordinarily low efficiency of incandescent lamps.

Penetrating Radiation. After the discovery of X-rays by Roentgen in 1895, a number of workers looked for penetrating radiation from various sources. The first person to test a lampyrid was Henry (1896), who covered photographic plates with "papier aiguille," placed glow-worms on them for periods of a half to two hours and observed after

development "black and white trails which reproduced almost exactly the route traversed by the ventral lanterns of this capricious animal."

Muraoka, again in 1896, covered photographic plates partly with cardboard and partly with metal and exposed them for two nights to glow-worms in a container, finding that on development the region covered by the cardboard had been affected. He thought the glow-worm emitted penetrating radiations but later Muraoka and Kashya (1898) discovered the effect was due to the cardboard itself.

As previously pointed out in connection with bacteria, the influence of many substances, cardboard, paper, gums, wood, and many metals (like Zn, Al, Cd) on a photographic plate often called the "Russell effect," is due to chemical action, in most cases peroxide formation. Metals like Al or Zn in moist air tend to dissolve in water, forming hydrogen and H_2O_2 , which affect the plate. Keenan (1926) has reviewed the extensive literature on this subject. Coblenz (1912) tested for presence or absence of X-rays from fire-fly lanterns by placing them near a Ba platinocyanide screen, which is readily excited by X-rays, and found no fluorescence of the screen. Penetrating radiation is certainly absent.

Phengodidae

The Phengodidae, according to the classification of M. Pic (1927), contain the genera *Pterotus*, *Phengodes*, *Baloscelis*, *Zarhipis*, *Mastinocerus*, and *Cenophengus*, with *Trachelychnus docens* of Bogota of uncertain position. Luminous forms are found in the genera *Phengodes* and *Zarhipis*, and it is likely that *Mastinocerus* and such additional genera as *Astraptor*, *Euryopa*, and *Ceratophengus* are also luminous.

Phengodes contains over thirty species, confined to North, Central, and South America. They appear similar to *Phrixothrix*, except they lack the red light. The record of *Phengodes* in North America begins with a note of Osten-Sacken in 1862 on unknown larvae which he thought might be lampyrids, telephorids or elaterids, probably the latter, of the genus *Melanactes*. Bethune (1863), Mann (1875), and Riley (1880) also thought them elaterid larvae but Riley (1887), after finding the "larvae" in coitus with males of *Phengodes laticollis*, correctly established their identity as larviform females of the Phengodini. Rivers (1886) described the luminous female of *Zarhipis riversi* from California, and Atkinson (1887) found that his female, 60 mm long, burrowed in the ground by day and came out at night, attracting the small males (15-20 mm long) with plumose antennae.

Since that time they have been observed and described by many

authors. Hayward (1898), Britton (1903), Knab (1905), Barber (1906, 13, 14), Knaus (1907), Calvert (1925), Harvey (1940), Owens (1944), Burbank and Lower (1946), and others have studied *Phengodes*. The author has seen only four living luminous specimens over a period of twenty-five years. In one, 60 mm long and 12 mm wide from Springfield, Missouri,²¹ there were twelve segments which contained yellow-green luminescent regions, either spots at the posterior lateral corner or transverse lines of light along the posterior margin. The three thoracic segments showed a transverse band of luminescence, broken in the middle. On the nine abdominal segments there was a transverse band and also a pair of luminescent spots, with the exception of the last abdominal segment (ninth) which lacked the band. It is not certain whether the above arrangement is the same for all species of *Phengodes*, whose classification is based on adult male characteristics. The Springfield, Missouri, specimen was an adult female, as the abdomen contained eggs. The eggs were not luminous, in this respect differing from the eggs of the fire-fly. The animal is shown in Fig. 147.

Another specimen from Tuxedo, New York, was cream colored and continuously luminescent day and night without any change in intensity. In other specimens the intensity of the light increased on handling. According to Buck (1950) the cream-colored individuals with continuous light are adult females while the brown pigmented forms are larvae. Their light may disappear for a period and may increase in intensity on stimulation.

When observed at night with a microscope the light of the spots and, less clearly, that of the bands is observed to come from small isolated luminescent dots, about ninety in each spot, which probably represent the luminous cells. As in the case of *Phrixothrix*, the luminescence disappears in absence of oxygen. Unpublished experiments carried out with Dr. F. H. Johnson indicate that the light suddenly returns with two to four times the intensity when oxygen is readmitted. The increased light immediately begins to decrease, and in about three minutes has resumed its original value. This "excess luminescence" after anoxia is quite comparable to that of luminous bacteria except for the time relations. The slow drop in luminescence intensity in *Phengodes* may be connected with the slow diffusion of oxygen to the luminous cells.

Buck (1950) was unable to observe the increased luminescence after anoxia in an adult female. He found that in nitrogen containing

²¹ Kindly presented by Dr. Wm. D. Burbank of Drury College, Springfield, Missouri.

0.25% oxygen, *Phengodes* becomes immobilized, and a just perceptible luminescence is emitted. With 1% oxygen, locomotion continues, but the glow is not as bright as that in air. In pure oxygen and air the light is of the same intensity.

When an animal is dark from lack of oxygen, the region of the previously luminous spots can be observed to be more fluorescent than

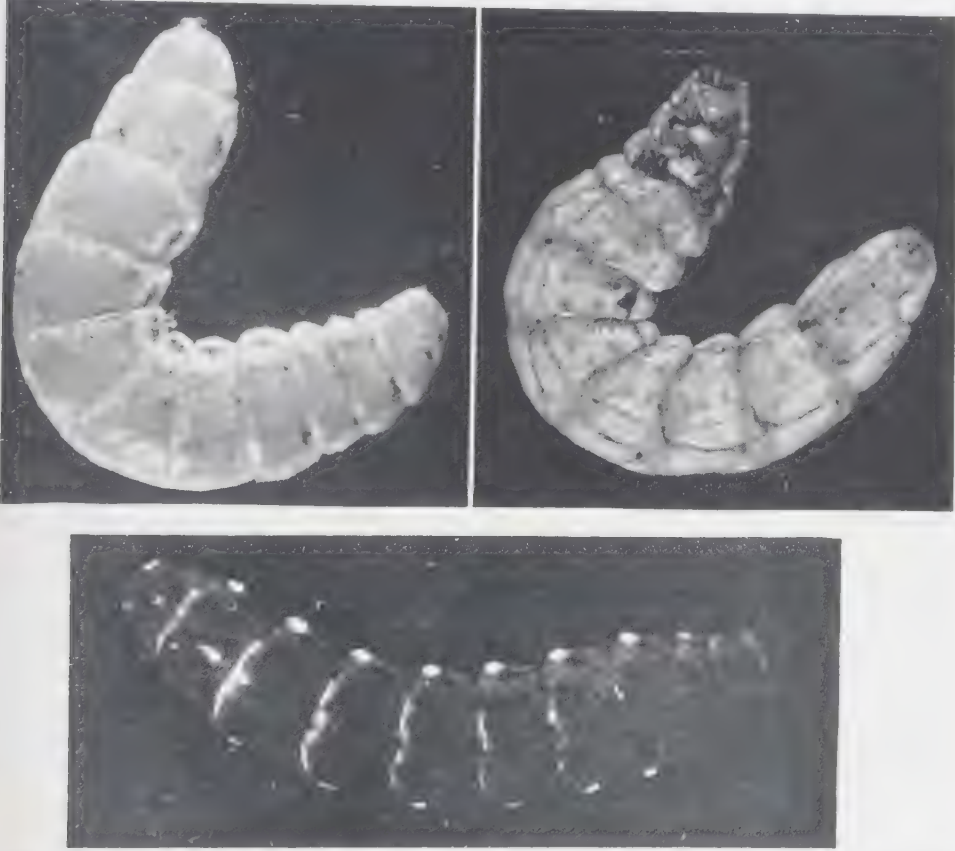


FIG. 147. *Phengodes* sp. photographed by daylight (dorsal and ventral view) and by its own light. Photos by G. Lower and W. D. Burbanck.

the surrounding tissue in ultraviolet light. This fluorescence is perfectly definite although not nearly as bright as the bioluminescence. Sometimes a small group of luminescent dots away from the main group is observed to be bioluminescent, as if the luminous cells had migrated, and these isolated luminous spots are weakly fluorescent also.

A histological study of the animal by Buck (1947, 48) has revealed that the luminescent dots are oenocytes or large specialized cells so similar to oenocytes that they cannot be distinguished from them. Groups of these cells are found in sections in the exact position of the spots and bands of luminescence. Each cell is oval, about $85 \times 60 \mu$

with considerable variability in size. Figure 148 shows the general character of the photogenic cells. In addition to these luminous oenocytes, other non-luminous oenocytes are found scattered through the body of *Phengodes*, especially between the branches of forking tracheae.

The luciferin-luciferase reaction has not been tested, and it is not yet known what the effect of ATP on extracts of the light organs may be.

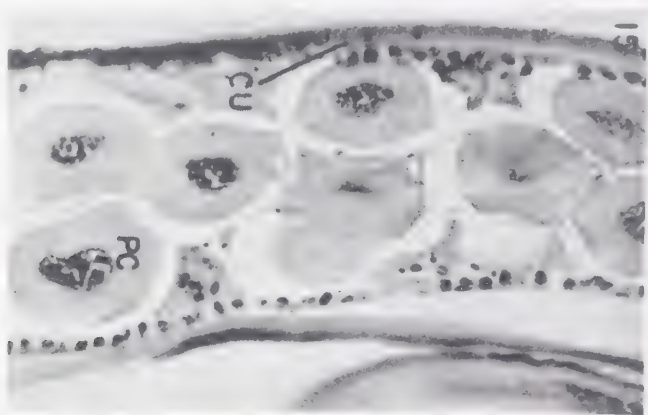


FIG. 148. Section of a lateral light organ of *Phengodes* sp., showing large luminous oenocyte-like cells, apparently slightly shrunken. After Buck.

Rhagophthalmidae

From time to time reports appear of luminous beetles or beetle larvae with many light organs, like those of *Phengodes*, found in tropical regions of the Far East. Green (1912) caught such a form, a male and female copulating, in the Peradenia Botanical Gardens, Ceylon, and identified it as *Dioptoma adamsi*. The "male *Dioptoma* (hitherto supposed to be non-luminous) displays—under sexual excitement—a brilliant series of lights of an emerald green colour. There is a transverse series of 4 luminous spots along the posterior margin of the prothorax; a marginal abdominal series of 8 on each side; and two converging dorsal series (of 3 points) on the hinder segments of the abdomen." The female *Dioptoma* was an elongate apterous grub-like insect with the "photogenic organ roundly quadrate, almost completely occupying the center of the penultimate segment; emitting an intense greenish-yellow light." In the same paper Green described the bioluminescence of *Harmathelia bilineata*, (now placed in the *Megalophthalmi* of the *Lampyridae*) although previously (1911) he had observed no light from this form.

Drilidae

The Drilidae, as classified by E. Olivier in 1910, contain some twenty genera of which a considerable number are known to be luminous, as indicated in the classification under Cantharoidea. The relationships of these genera are not too well understood and some luminous drilids have been placed in other families—Dodecatoma in the Rhagophthalmidae, Cydiscus in the Phengodidae, and Telegeusis in the Lymexylonidae. The best-known luminous genera are Diplocladon and Phrixothrix. The former is from the Far East, the latter from

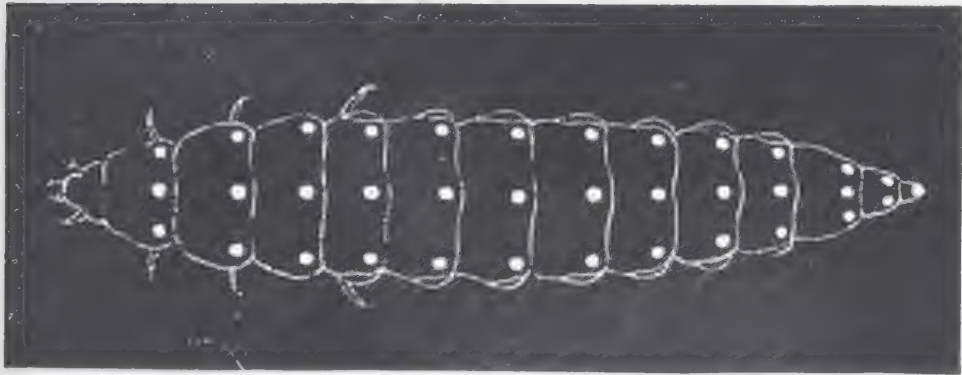
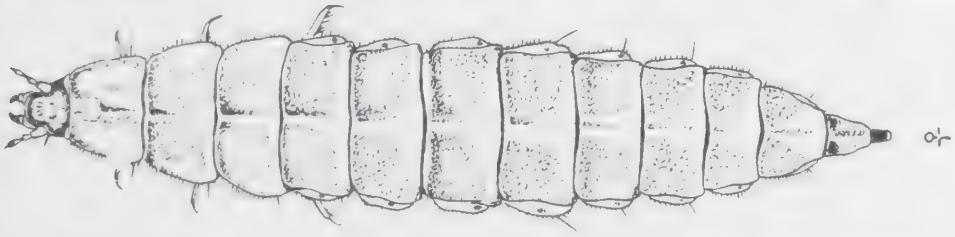


FIG. 149. *Diplocladon Hasseltii*, female, the "star-worm," indicating the position of luminous organs. Drawing by Y. Haneda.

Central and South America. In both genera the males are winged and the females wingless and larviform.

Diplocladon, the "Star Worm." These insects are quite common in the Singapore region and are also found in British North Borneo. Such a specimen was probably caught by Gahan (1924), who described a large glow-worm in Malaya with three lights on each segment of the body forming three longitudinal rows of lights, one central and two lateral. Recently Haneda (1950) has collected many star worms in Singapore and found one copulating with a male. The male has been identified by the late H. S. Barber²² of the U.S. Bureau of Entomology

Barber was preparing a revision of the Drilidae and related families at the time of his death in 1950 and contemplated placing *Diplocladon* in a new family, near the Phengodidae.

as *Diplocladon Hasseltii*. The female and larva resemble a *Phengodes* except for the middle line of luminous spots. The three thoracic and eight abdominal segments carry three light organs, the ninth abdominal or penultimate two light spots, and the last segment one median spot as shown in Fig. 149. The luminescence is continuous and greenish blue. Indeed, the insect must be a striking sight and literally a star worm or a diamond worm, "ulat intan," as it is sometimes called in Malay. Photographs of both male and female are reproduced in Fig. 150.

One of the earliest records of a *Diplocladon* or an allied larva was published by Waterhouse (1889), the description of a 1.5 in. specimen received by the British Museum from Hangchow, China. The animal

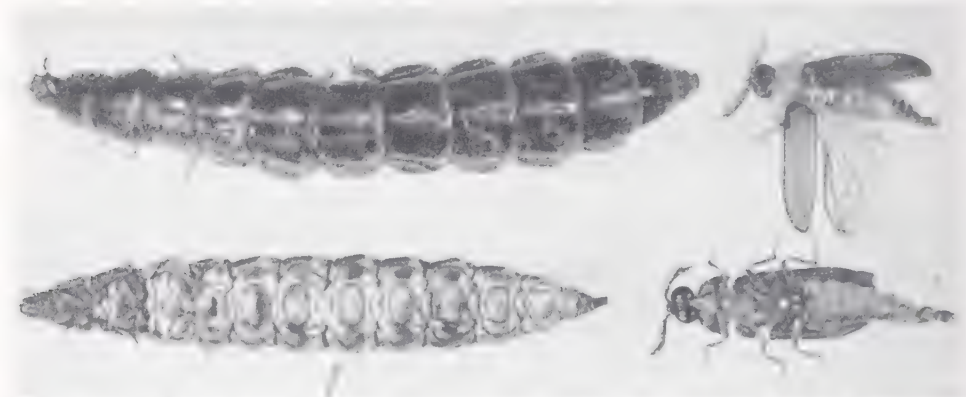


FIG. 150. *Diplocladon Hasseltii*, showing upper and under side of both female and the small male (to right). Photograph by U.S. National Museum.

sometimes attains a length of 2 in. There were three lights on each of eleven segments. These glowed continuously day and night and, according to the account, for three days after death. Handling had no effect on the light intensity. These specimens must resemble glorified *Phengodes* with an extra row of lights down the middle of the back.

Swinton (1880) has listed a *Drilus flavescens* among other luminous insects and it is possible that the larva found by M. Payie near Bangkok, Siam, 70 mm long and 20 mm broad that looked like a myriapod belonged to the Drilidae. It was described by Lucas (1887).

Phrixothrix, the "Railroad Worm." Specimens of *Phrixothrix* are perhaps the most remarkable of all luminous forms. The genus, which is placed by some in the *Phengodidae*, appears to be widely distributed over Central and South America. Through the kindness of South American friends, the author has had the opportunity of examining 13 living specimens, 9 from Montevideo, Uruguay, and 4 from São Paulo, Brazil.

Like fire-flies, *Phrixothrix* emits a greenish yellow light, but unlike

most fire-flies, the luminous spots are arranged in eleven pairs on the posterior lateral margin of the second thoracic to the ninth abdominal segments of the body, with an additional red luminous area on the head. In darkness, when the red region alone is shining, the animal looks like a glowing cigarette.

The adult female is larviform, and the above arrangement of luminous spots is restricted to larvae of both sexes and adult females. The



FIG. 151. Photograph of *Phrixothrix*, the "railroad worm," by flash bulb. By LIFE Photographer Fritz Goro (c) Time, Inc.

adult male has the typical beetle form, with long-branched antennae and no definite luminous spots. However, a diffuse yellowish glow of the adult male appears on stimulation from eight of the abdominal segments, although no red is to be observed. Photos of the railroad worm are reproduced in Figs. 151 and 152, and an adult male is shown in Figs. 153 and 155.

The various specimens received by me showed no light when at rest but if disturbed very slightly by knocking the table gently, or blowing

air over them, they responded by shining the red light. When the disturbance was greater the rows of yellow green lights appeared and the animal explored its environment with a brilliant display of pyrotechnics. Sometimes all and sometimes only certain segments with greenish lights would be turned on. Later the greenish lights went out while the red remained on for some time, finally to disappear as the animal became quiet again.



FIG. 152. Photograph of Phrixothrix by its own light. By LIFE Photographer Fritz Goro (c) Time, Inc.

History. It is not surprising that such a wonderful display of luminescence should excite the interest of travelers. Azara (1809) is usually credited with the first record of the insect, seen in the last decade of the eighteenth century, but a passage in Oviedo (*Coronica de las Indias*, lib. 15, cap. 2, fol. 113) may mean that the railroad worm was known much earlier. After describing luminous myriapods found on the island of St. Domingo, Oviedo says "There are others which in all that has been stated, are like these [myriapods] in size and in shining, but they have this great difference, that the head also shines, but the light of the head is like that of a very bright burning coal."

Quoted from G. Newport, *Trans. Linn. Soc.*, 19, 431, 1845.

Reinhardt (1854) gave a careful description in Danish of a specimen from Lagoa Santa found in April, 1853. In 1868, Murray named the animal *Astraptor illuminator*, deriving the generic name from the Greek, *astrapton*, meaning a flash of lightning. This specimen was found near Rio de Janeiro and described as "No. 368—Rio. Red light in head, white light in the tail and one light on each side on each segment of the body. Light in the head permanent, the others showing by flashes." Murray thought the light was due to chemical action, i.e., a combustion throughout the body which was visible through the spiracles. Actually the light does not come from the spiracles but from small organs posterior to them.

Burmeister (1872) observed a similar insect, caught in 1858 in rotten wood near Paraná, the former capital of the Argentine Republic. It was 2 in. long and $\frac{1}{4}$ in. wide and ejected "from the anus a clear reddish brown fluid which had a corrosive effect upon the skin. During all this time it was emitting light. . . . This light, which the animal can intensify or diminish at will is of two different colors. At the head end it emitted an entirely red light like a burning coal; but on the body the light was greenish white, like that of the glow-worm, or of phosphorus."

Several additional records and descriptions of "*Astraptor*" by Smith (1869), Trimen (1870), Weyenbergh (1874), Jhering (1887), Gahan (1907), and Barber (1908) are to be found in the literature. Most of the early observers thought that the insect was the larva of the elaterid, *Pyrophorus*, but in 1886 Haase described "*Ein neuer Phengodes*" and in 1887 published a long paper giving the history of previous observations and a detailed description of a pair of the insects caught in copula by Dr. Hieronymus at Cordoba on October 10, 1881. This lucky find established the true identity of the larva as a *Phengodes*-like insect, named *P. hieronymi* by Haase (1886). His drawing is reproduced as Fig. 153. In all species of this genus the adult females, pupae, and larvae are hardly distinguishable from each other while the males are normal winged beetles. Dr. Hieronymus kept the living fertilized female, which laid eggs that later hatched to larvae during the last part of December. The eggs were not luminous, but the larvae (11 mm long) had red and greenish lights like the mother. The adult male showed a greenish light from the underside of the abdomen. The year before Haase's paper appeared, Dubois (1886) in his monumental work, *Les Elaterides Lumineux*, had figured the larvae described by Murray and Burmeister and pointed out that they differed greatly from the larva of *Pyrophorus*.

Biology. Much information regarding the biology of *Phrixothrix*

has come in letters from Dr. H. L. Parker, of the U.S. Department of Agriculture, stationed in Montevideo, to the author. The animals come out at twilight in the spring and are best obtained by following the plough as fields are prepared for planting. Parker wrote "The food of the insects is white grubs. . . . When a grub is attacked, the ferrocarril pours quickly into the grub a colored salivary juice that spreads rapidly throughout the body turning it blackish in a few minutes."

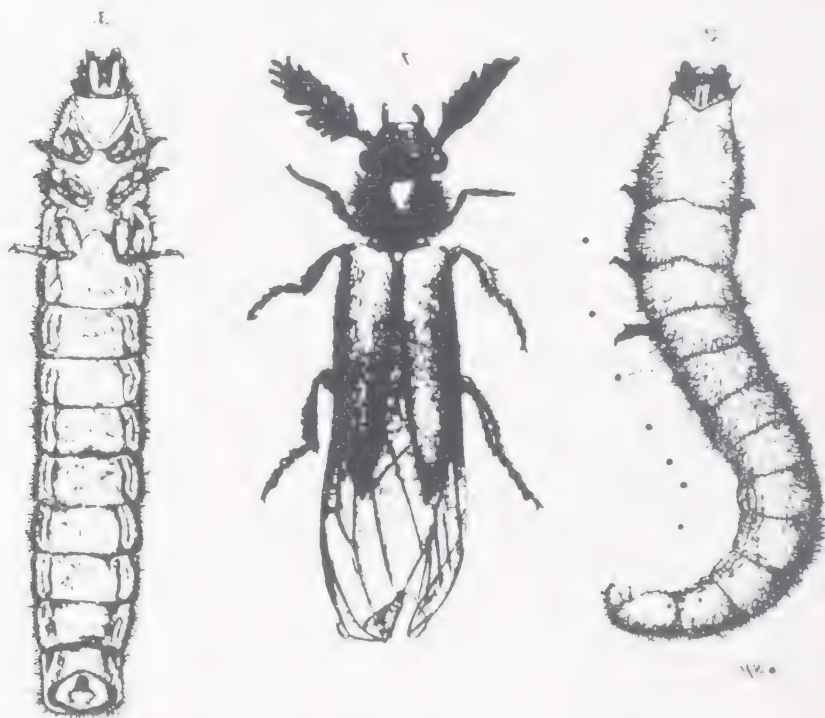


FIG. 153. The original drawing of *Phrixothrix* by Haase in 1888. Left, under surface of female, showing light organs. Center, male. Right, upper surface of female.

After referring to a large catch of male *Phrixothrix* near Colonia Suiza, Uruguay, Parker says "There are two species of these insects, one (the one evidently described as *Phengodes heironymi* by Haase) has the thorax yellowish and the branches of the antennae rather long; the other has the thorax black, wider than the eyes and the rami of the antennae relatively short. They are both luminous in the adult stage. . . . These males give off a feeble yellowish light (not greenish as does the larva) from apparently eight abdominal segments. Ordinarily they do not light up, but if taken in the fingers and squeezed slightly the light will flare up, remain light a short time and subside.

"I have procured a few eggs from one female ferrocarril but do not

seem to be able to maintain the proper conditions for hatching them, practically all have shrunk and broken. The eggs do not emit light.

The female adult of one species (the smaller one), although maintaining the larval form, loses eight lights on each side but maintains the red light in the head and three yellowish-green on each side posteriorly on the abdomen. All lights are less brilliant, and it is more difficult to induce the female to 'light up' than the larval stage.

"I cannot distinguish the female adult from the larva, although the female pupal stage had blunter and lighter colored mandibles, triangular in shape, and the spiracles are simple while the larval spiracles are biforian and have a horse-shoe shaped sclerome under them."

Histology. When examined with a microscope, the author (1944) could not make out separate photogenic cells through the integument of living *Phrixothrix*, as is possible in *Phengodes*. The light in both red and green organs appears to be homogeneous. Even when dissected out no separate luminous cells could be observed, and when the luminescence had disappeared in absence of oxygen, no marked fluorescence revealed the presence of luminous regions such as can be seen in *Phengodes* in ultraviolet light.

The histology of the luminous organs has been studied from sections by Buck (1947, 48), who found the lateral yellow luminescent spots to be made up of a small group of photogenic cells forming an ovoid mass quite similar to the thoracic light organs of *Pyrophorus*, or to that of the larvae of fire-flies or of such adults as the European glow-worm, *Lampyrus*, and the genus, *Diphotus*, among others. These yellow-green luminescent organs of *Phrixothrix* are supplied by a tracheal trunk that ramifies to smaller and smaller branches which end among the photogenic cells. No tracheal end cells are present, in this respect agreeing also with the light organ of the above mentioned forms. Such an arrangement seems to be characteristic of luminous beetles in which the light remains on for a considerable time, appearing slowly and fading out slowly. It is to be contrasted with the flashing lights of true fire-flies, where the tracheal distribution is complex and tracheal end cells intervene before the tracheolar branches reach the photogenic tissue. A section of the yellow organ of *Phrixothrix* is reproduced as Fig. 154.

The red light organ in the head has also been studied by Buck.²⁴ It has the same general structure as the lateral organs and appears to be made up of at least four more or less distinct parts.

Biochemistry. With living specimens it has been possible to settle two important questions concerning the nature of the colored light

²⁴ Private communication.

(Harvey, 1944, 45). First, the red luminescence is due to emission of red light and not to a red filter absorbing other wave lengths, since it is very apparent when the head is dissected open and the transparent luminous material observed directly without the somewhat brownish chitinous cuticle. Secondly, the red light is not the red fluorescence of a substance excited by shorter wave lengths of some other chemiluminescent reaction. This has been determined by exposing the animal to near ultraviolet light without the visible. Although such light is most active in exciting fluorescence of many compounds, no red fluorescence



FIG. 154. Section of a lateral light organ of *Phrixothrix*. P, photogenic tissue; FB, fat body; T, trachea; CU, cuticle. After Buck.

could be detected when the red bioluminescence of the organ was not showing, or if it had been purposely suppressed by placing the animal in an atmosphere of hydrogen or nitrogen, or after the organ had been macerated and the red bioluminescence had disappeared. In addition no fluorescence appeared when the head was irradiated with blue, green, yellow or red light. There is also very little yellowish fluorescence of the greenish-luminescent organs of *Phrixothrix*.

The fact that the bioluminescence of both red and green organs of *Phrixothrix* disappears in nitrogen or hydrogen indicates the importance of oxygen, as in the majority of bioluminescences. It was also determined that the red luminescent material dissected out of the animal emits no light in a hydrogen atmosphere. This experiment shows that the disappearance of light is due to removal of oxygen from

the luminous material rather than to a reflex response of the animal to lack of oxygen, resulting in inhibition of luminescence. The necessity of oxygen, together with the very definite absence of red fluorescence from the red luminescent region of *Phrixothrix* indicates that the red emission is a red oxidative chemiluminescence such as can be obtained from oxidation of Mg and Zn complexes of certain porphyrins, phthalocyanines, and chlorophyll derivatives.

The red fluorescence of chlorophyll and porphyrins are well known, and it would not be surprising to find red fluorescence of a porphyrin derivative in a luminous organ which emits red light. However, the combination of porphyrin derivatives with proteins are not always fluorescent. Using fluorescence as a test the evidence against a porphyrin in the red bioluminescent regions of the head is therefore not unequivocal. All attempts to detect a visible red or green pigment in the region of the head from which the red bioluminescence is emitted have also failed. The tissue is clear and transparent. If such a pigment is present, its concentration must be too low to be visible in a thickness available for eye observation, but again the evidence against a red pigment is not unequivocal.

On the other hand, an intense red pigment does exist in the body of *Phrixothrix*. As already mentioned, Burmeister (1871) had observed this pigment to be extruded from the anus when the animal was roughly handled. On dissecting the animal, the last third of the intestine, posterior to the entrance of the two Malpighian tubules, is found to be filled with a bright red oily fluid. This liquid sticks to paraffin and rises as globules to the surface of Ringer's solution without readily mixing with it. The red pigment is probably produced in the Malpighian tubules. It frequently covers the outside of the anterior segments of the body because of the strong tendency for the larva to coil up with tail end near head end, but it is completely absent within the head where the red light is emitted.

When mixed with water the red color becomes more yellowish and on standing turns brown. When absorbed on cotton or filter paper, the initially red spot also turns brown. However, the concentrated red oily liquid will remain red for some time in a moist chamber. It exhibits no definite absorption bands and in this respect the red intestinal pigment appears quite unrelated to porphyrins. In dilute solution, there is no marked fluorescence in ultraviolet light, but the concentrated material newly removed from the gut is red fluorescent. More material will be necessary to establish the class of pigments to which it belongs. The red pigment might serve as a reservoir of some luminescent compound or some precursor which is supplied in low concen-

tration to the proper enzyme of the head and oxidized with light emission. However, such a mechanism has yet to be proved and seems in the whole to be rather improbable.

In a single experiment, two animals were sacrificed in an attempt to demonstrate the luciferin-luciferase reaction. The solutions of these substances were prepared in the usual way; luciferin by a hot water extract of the greenish luminescent organs allowed to cool and luciferase by a cold water extract which had stood until the luminescence disappeared. When the two dark solutions were mixed, no luminescence appeared. A similar experiment with the red luminescent organs also gave negative results. However, because of the small supply of *Phrixothrix* material, these negative results might be attributed to low concentration of photogenic substances and must not be considered conclusive.

The close relationship of *Phrixothrix* and lampyrids suggests that adenosine triphosphate (ATP) might be concerned in light production. In two experiments on specimens of *Phrixothrix* received from Dr. Paulo Sawaya of the University of Sao Paulo, Brazil, the author (1949, 50) found that no light appeared on addition of ATP to dark extracts of either yellow or red luminescent organs. However, when purified fire-fly (*Photinus pyralis*) luciferin, kindly supplied by Dr. W. D. McElroy, was added, luminescence appeared from the yellow luminescent organ extract. Fire-fly luciferin added to the red luminescent organ extract gave a very faint, if any, light. The experiments suggest that the yellow luminescent organs behave like those of the certain fire-flies in which luciferin quickly disappears on making an extract of the lanterns. Confirmation of the behavior of the red luminescent organ must await additional specimens of *Phrixothrix*.

ELATEROIDEA

Elateridae

Insects with luminous organs of great intrinsic brilliancy are found among the click beetles, the Elateridae. They belong in the subfamily, the Pyrophorinae,²⁵ containing eleven genera, three of which are luminous: *Pyrophorus* with one hundred or more species, restricted to tropical and subtropical America; *Photophorus* with two species, *P. bakewelli* and *P. jansonii* from the New Hebrides and Fiji Islands, respectively; and *Campyloxenus pyrothorax* from Chile. Certain species of *Pyrophorus* are found in Texas and Florida (Moore, 1873; Monzetti

²⁵ See W. Junk *Coleopterorum catalogus*, Vol. XI, Elateridae, Part II, by S. Schenkling, pp. 345-56, 1927.

1921). The non-luminous genera are *Alampes*, *Alampoides*, *Hifo*, *Hifoidea*, *Paraphileus*, *Meroplinthus*, *Phryschi*, and *Heligmus*.

Almost every traveler to the Caribbean region has mentioned luminous elaterids, which Spaniards call "cucujo," and the French, "taupin," shown in Fig. 155. Oviedo, Martire, Dutertre, Stubbes, Norwood, Sloane, Brown, Azara, Humboldt, Spix and Martius, and others too numerous to mention have recounted the marvels of the

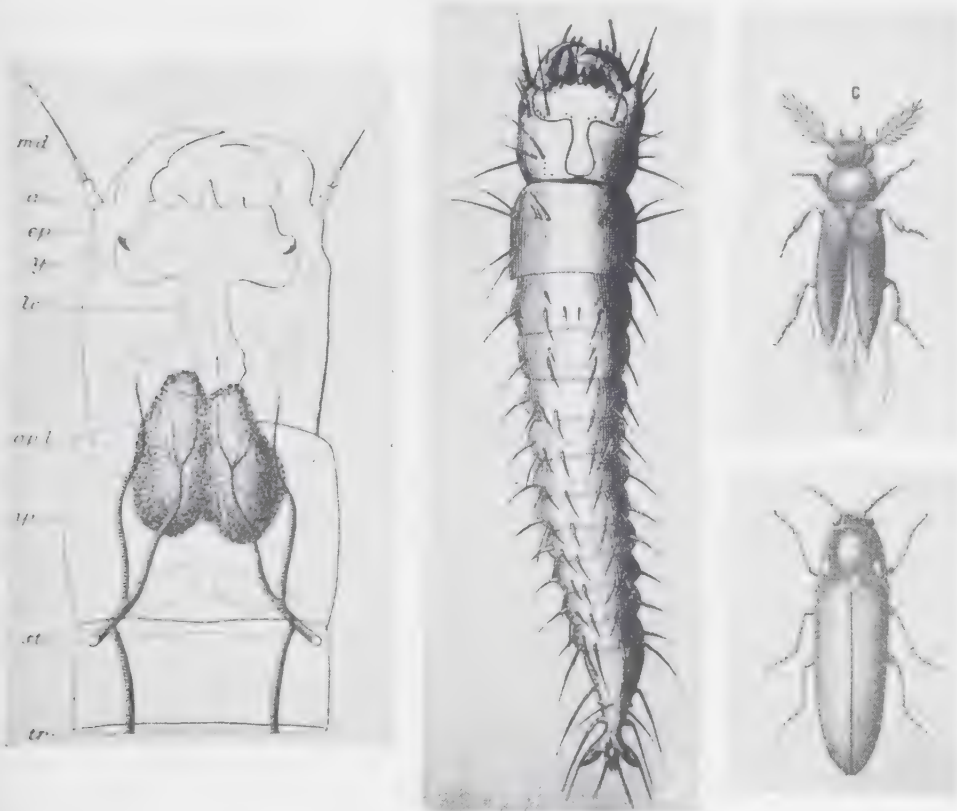


FIG. 155. Left, enlarged view of head of a *Pyrophorus* larva showing luminous organ; center, the larva itself, after Dubois. Right above, *Phengodes laticollis*, male; below, *Pyrophorus noctilucus*, adult, after Hennequey.

cucujo and its use for illumination, for ornament or in play. Fougereux de Bonderoy in 1766 described a *Pyrophorus* which was found in the Faubourg St. Antoine in Paris, evidently escaped from some shipment from Cayenne. All the early naturalists (Aldrovandi, Mouffet, Nierembergius, Linné, DeGeer) included the animal in their writings, as did the text books of entomology. Macartney (1810) treated *Pyrophorus* in his *Observations on Luminous Animals* and gave a figure of the insect, so great was the interest in these forms.

More or less popular accounts have been written by Curtis (1827), Gosse (1848), Perkins (1869), Gerard (1873), Hermanas (1874), Beach (1874), Bowles (1882), and Boyer (1934), and a number of scientific studies have made the anatomy, physiology, and histology well known. In addition to the numerous short accounts of Pyrophorus light, two great monographs on the insect have been published—both in 1886—one by Heinemann and one by Dubois. Heinemann had previously (1872, 73) published on the chemistry of the light organ and his monograph is largely physiological.

The work of Dubois, *Les Elaterides Lumineux*, is more comprehensive. It deals exhaustively with the history (with a bibliography), anatomy, histology, embryology, physiology, and chemistry of the organ, as well as the physical properties of the light. Dubois's interest started with a specimen received from a ship at Le Havre, which led to a study of the spectrum and eventually to the monograph.

Pyrophorus and also Photophorus possess two kinds of luminous organs, similar in structure. The most obvious is a pair of greenish luminescent oval spots, which look like eyes, on the posterior lateral margin of the prothorax. These brilliant spots of light have the appearance of auto headlights, and the insect is sometimes called the "automobile bug." In addition there is a ventral, irregularly heart-shaped, orange luminescent organ on the first abdominal segment, visible only when flying or when the elytra are expanded. Adults are frequently found in the cane fields where they suck the sweet juices from bruised portions of the cane. The females deposit eggs in dead wood and humus. According to Dubois (1886) the egg is diffusely luminous when laid and the newly hatched larva is like other elaterid larvae except for the median bilobate luminous organ between head and first thoracic segment, as shown in Fig. 155. In later larval stages other light organs appear. They have been studied by Dubois.

As in the case of fire-flies, Pyrophorus is nocturnal, hiding under the bark of trees or leaves during the day, becoming active at dusk and flying throughout the night. The light is probably used to attract the sexes, although no special study of this point appears to have been made. Blair (1926) has reported observations of Dr. C. L. Withycombe in Trinidad, who once saw a male follow a female in a straight line, whereas the usual flight is slow and irregular. Both individuals landed on the ground, and the female extinguished her light. The male apparently found her by the sense of smell. The light organs have the same form in both male and female, differing in this respect from fire-flies, where sexual dimorphism is common.

Histology. Fine structure of the adult light organs of *Pyrophorus* has been studied by Heinemann (1872, 86), Robin and Laboulbene (1873), Dubois (1886), Geipel (1914), Dahlgren (1917), and Buck (1948). Both prothoracic and ventral organs correspond to the type 3 organs of lampyrids, having the photogenic cells at the surface and the reflector layer cells, filled with urates or guanine (Dubois) underneath the photogenic layer. There are no tracheal end cells but large tracheal trunks supply air through branches which terminate between the photogenic cells. Figure 156 shows the general structure of the organ.

While the nocturnal genus *Pyrophorus* possesses luminescent spots on its prothorax, the diurnal non-luminous *Alaus oculatus* has two

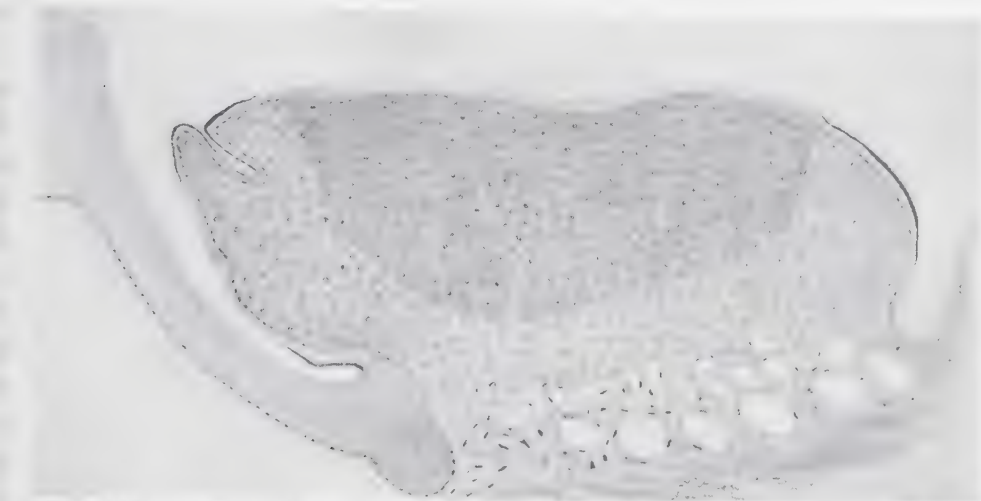


FIG. 156. Vertical longitudinal section of the abdominal light organ of a male *Pyrophorus*, showing darker photogenic cells, surrounded by lighter reflector layer. After Dahlgren.

conspicuous black spots in that region but on the middle rather than the posterior-lateral edge. McDermott (1911) suggested the black spots might indicate an evolutionary trend toward luminosity but was unable to find anything in sections that resembled luminous tissue.

Physiology. The light of *Pyrophorus* appears slowly, gradually rises to a maximum, persists for a considerable time and then gradually fades, corresponding to the type of lighting characteristic of lampyrids without tracheal end cells. It is definitely under the control of the nervous system. Special physiological studies have been made by Heinemann (1872, 86), Dubois (1886), Fuchs (1891), and Harvey (1931), and occasional observations by Robin and Laboulbene (1873).

The insects do not light spontaneously during the daytime unless handled or gently squeezed. After such stimulation, the light of the prothoracic organs first shows in the center and spreads toward the periphery. When the organs darken, the center may darken first and then the periphery. According to Heinemann this behavior depends on the position of tracheae entering the organ.

Both Heinemann and Dubois endeavored to connect the lighting of *Pyrophorus* with breathing movements or circulatory changes. Heinemann distinguished two kinds of light (1) "cell light," a soft glow sometimes observed in quiescent ("sleeping") insects or in excised organs and (2) "tracheal light," the bright luminescence of active and breathing animals. He held that control was by respiratory muscles and that the abdominal organ might glow with each respiration, but such a correspondence did not always take place. According to Heinemann, the abdominal organ has a special tracheal system and glows on inspiration when air is suddenly drawn in and on expiration (the active process in insects), due to muscular contraction, which forces air into the fine tracheoles of the organ. When a *Pyrophorus* was cut in two behind the prothorax, Heinemann found that stimulation of the ventral nerve cord would either cause the abdominal organ to glow or a glowing organ to cease luminescence. He believed these effects depended on whether the stigmata were closed or open. If closed, light could appear on muscle contraction due to increase in pressure; if open the light would cease because all air would be forced out of the tracheal system by contraction of body muscles.

The mechanism of lighting in the prothoracic organs was not so clear. Heinemann cut off the head and found that stimulation of the ventral nerve cord caused luminescence, but he could obtain no light if the muscle mass of the prothorax was stimulated. Nevertheless Heinemann felt that the prothoracic organs should be dependent on air supply and in favor of this view he found that if air was forced into the prothorax by a glass tube inserted in a stigma, a bright light resulted. This behavior is quite similar to the light increase of fire-flies when the pressure is increased, and it is very doubtful if breathing movements have anything to do with the lighting of a *Pyrophorus*.

Dubois in fact came to the conclusion that respiratory movements were not responsible for the initiation of luminescence in *Pyrophorus* nor for the pulsations of light intensity sometimes observed. He thought respiration exerted only an indirect influence by maintaining the tissues and blood in good physiological condition. He considered that heart beat also affected the light only in so far as it supplied oxygenated blood to the cephalic ganglion, but did believe that nerves

stimulated muscles which in turn supplied more blood to the light organ, thus initiating the light. This view is also incorrect. Fuchs has shown that special nerves go directly to both prothoracic and ventral organs. When stimulated electrically, luminescence results.

Since the prothoracic organs are readily accessible without injury or

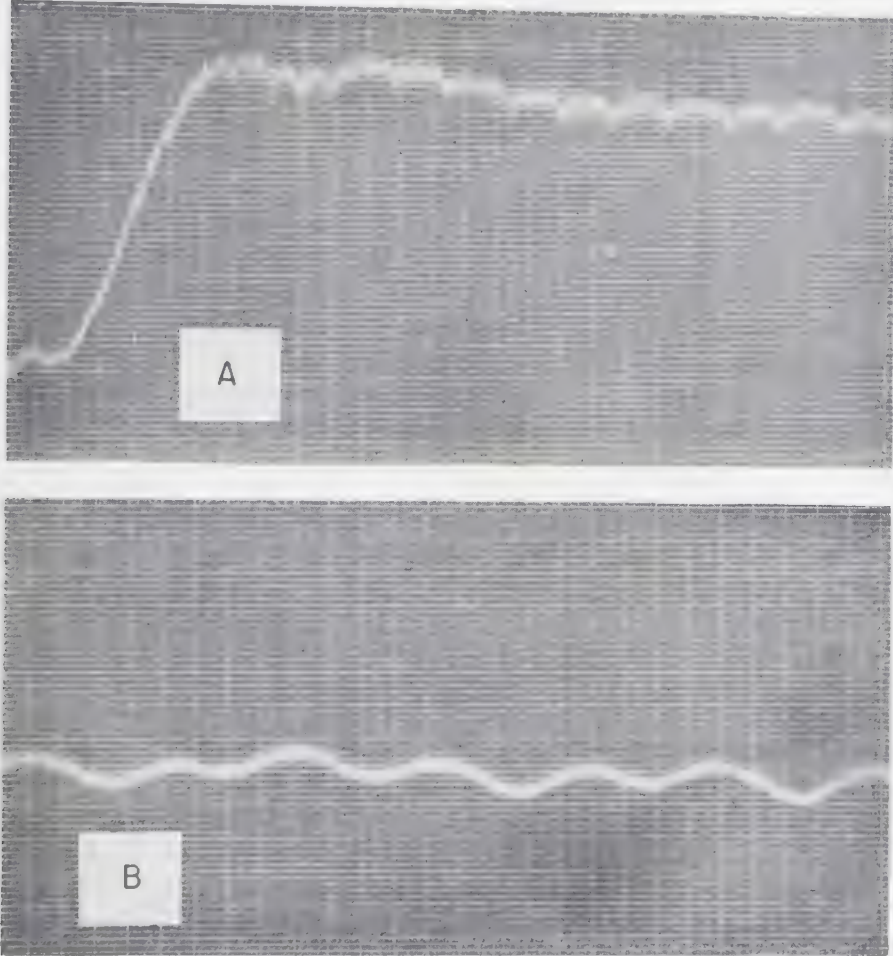


FIG. 157. String galvanometer record of the light from prothoracic organs of a *Pyrophorus*. A, beginning of light emission; B, the same record after 15 seconds. Light intensity vertical in arbitrary units. Time between lines 0.1 second. Original record.

disturbance of the insect, they offer ideal material for study, and the author has recorded by photocell-amplifier-string galvanometer technique the normal reflex response to stimulation by squeezing the abdomen. The luminescence which results appears quite steady to the eye, sometimes varied with an occasional fluctuation in brightness. If squeezed more forcibly, but after the light has nearly subsided,

rhythmic fluctuations in intensity occurring at intervals of 0.8 to 1 seconds may be observed. This rhythmic fluctuation amounting to 25% of the total light emitted, can sometimes be observed when the animals are walking around in a dish in a dark room. Records are reproduced in Fig. 157.

In addition to the pulsation, the records show small rapid fluctuations in intensity, representing a change of some 5% of the total light emitted which cannot be detected by eye but are readily picked up with a photocell. They also are shown in Fig. 157. It will be observed that 0.8 to 1 second is taken for maximum brightness to be attained and that the rate of increase in brightness is more or less linear over the greater part of its course, only the beginning and end showing deviations. The striking phenomenon is the rhythmic change in light intensity, which sometimes starts at a rate of 300 per minute but more frequently at 214 per minute, falling off after 20 seconds to 150 a minute.

To make sure that the Pyrophorus light is not made up of individual flashes of even more rapid rhythm, a beetle was fixed on a centrifuge head of 26 cm diameter and observed in a dark room while the speed of revolution was increased gradually up to 3000 rpm. At no speed did the luminescence appear anything but continuous. Since the animal traveled 400.00 mm per second at the highest speed, a rapid rhythmic flashing should have been easily detectable. Therefore the light of Pyrophorus is truly continuous (except for the rhythm displayed in the records) although it must be borne in mind that if the individual cells flashed intermittently at a rapid rate and were out of phase, the integrated result would be a continuous light.

There are four possible explanations of the 200 per minute rhythm, apart from vibration artefact, which was ruled out by proper experiments. It may be connected with the (1) click reflex, (2) respiration, (3) heart beat, or (4) rhythmic nerve discharges to the organ. First, when squeezed between the fingers these beetles click rhythmically at a rate of about one per second. The change in position of the thorax during this movement cannot be the explanation of the rhythm because the rhythmic variations in light intensity are too rapid and the record of each variation would be different, a sudden change in light intensity followed by a slow recovery, as can be proved by actual records of the light while clicking is occurring.

Second, the respiratory rhythm might force more air into the organ. However, the rhythm of breathing is much slower than the light rhythm exhibited in the records, a maximum respiratory rate of fifteen per minute after removal of the elytra and wings. Dubois' records

show 10, 20, and 24 respirations per minute and in addition the cessation of respiration when the prothoracic organs light.

Third, it is very unlikely that heart beat should influence luminescence intensity, since oxygen is carried to the light organ by tracheae directly and not through the blood. In addition, rate of heart beat is slower than the rhythm of luminescence. Dubois found that the heart rate is 106 per minute, dropping to 60 to 70, and the author has observed a rate of 84 per minute at 28° C after removing the elytra and wings, but leaving the tergites uncut.

Fourth, the most likely explanation is a rhythmic discharge from the nerve center for the organs, a volley similar to that sent out by many central ganglia. The records of Fig. 157 look very much like an incomplete tetanus of muscle and may be attributed to the same cause, rhythmic stimulation.

Temperature and Pressure. Dubois (1886) observed that *Pyrophorus* would luminesce between 0° and 47°, but no special study has been made of the intensity at different temperatures. Dubois also subjected *Pyrophorus* beetles in water to pressures of 600 atmospheres for ten minutes and found that they were non-luminous when removed. Although they survived two or three days, the light did not return. On the other hand, when he subjected dried and moistened prothoracic and ventral organs to the same high pressure, they were luminous on removal from the pressure chamber.

It is possible that a day-night rhythm of luminescence exists in *Pyrophorus* but no such study has yet been made. Heinemann (1872) observed inhibition of luminescence by light, and the author (1926) noted an effect on the intact prothoracic organs. They behave in a manner somewhat similar to the lantern of the fire-fly in a bright light. When a prothoracic spot of *Pyrophorus* is exposed to sunlight through glass for two minutes and the other kept in shadow, the luminescence of the light exposed spot dims as compared with that in shadow, with recovery in the dark. No study of the mechanism of this inhibition has been made.

Biochemistry. Macartney (1810) mentioned finding a yellow substance composed of globules in the light organs of *Pyrophorus*, but little was done on the chemistry of the light organs until after 1870. Robin and Laboulbène (1873) noted that the crushed cells contain a substance which behaved like noctilucine, the nitrogenous coagulable material obtained by Phipson (1871) from luminous centipedes, fish, etc., and Heinemann (1872) found that the material was acid to litmus. He (1873) also analyzed the ash of the ventral organs of 186 large cucuyos

and obtained positive tests for Cl , PO_4 , K , Ca , and CO_2 . There was present in the reflector layer K or Ca urate, but not NH_4 urate, as K  lliker (1864) believed. Dubois has called the reflector layer material guanine.

The idea that luminescence might result from oxidation of fat started with Panceri's researches on marine invertebrates and received considerable encouragement after Radziszewski's observations on the chemiluminescence of oils in 1880. The photogenic cells of *Pyrophorus*, like those of the fire-fly, do contain small granules that superficially resemble fat and Lund (1911) fed *P. plagiophthalmus* on sugar cane impregnated with the fat-staining dye, Sudan III, to determine if the granules would stain red. He found that the fat body stained deeply orange red but that the luminous organ was only pinkish, a little stronger than other tissues. The granules were not true fat and stained in a number of fat insoluble dyes (orange G., methyl green, acid fuchsin, gentian violet, erythrosin, and phosphomolybdic hematoxylin) with iron alum as a mordant.

Luciferin-Luciferase Reaction. As early as 1885 Dubois had found that the luminescence of *Pyrophorus* resulted from the action of a soluble ferment, coagulated by heat and spoken of as a diastase, acting on a heat-resistant substance. He showed that if the luminous organ was dipped in hot water, the light disappeared and did not return again; also if the luminous organ was ground up, the mass would glow for some time, but the light finally disappeared. When the previously heated organ was brought in contact with the unheated triturated organ, light again appeared.

Although this is the luciferin-luciferase reaction, the names lucif  rase and lucif  rine for these substances were not mentioned in the 1885 paper or in the monograph (1886) on *Pyrophorus*. They first appeared (Dubois, 1887) in connection with a chemical study of luminescence in the mollusc, *Pholas dactylus*, but there can be no doubt of Dubois' conviction that the photogenic mechanism in *Pyrophorus* and *Pholas* was the same.

Dubois also observed that the tissue could be dried and would light on moistening and that oxygen was necessary for luminescence. *Pyrophorus* has thus served as the luminous animal for the first important knowledge of enzymes in luminescence.

Adenosine Triphosphate. Recent work (McElroy and Harvey, 1950) has indicated that adenosine triphosphate (ATP) is also involved in the light production. If either the ventral or prothoracic organs are ground with water in a mortar and the extract is allowed to stand until the light disappears, addition of ATP will revive the light provided it is

added immediately. After a wait of twenty to thirty minutes, the ATP has no effect, in all probability because of the disappearance of a transphosphorylase in the extract.

Fluorescence. The fluorescent substance pyrophorine, later called luciferesceine, was observed in the blood of *Pyrophorus* by Dubois (1886, 1907). He also noticed (1907) that the luminous tissue fluoresced in the ultraviolet region of an electric arc spectrum although it was a little more "greenish" than the bioluminescence. The author has observed this yellow fluorescence of the luminous organs, which is very striking in the ultraviolet. The fluorescence color is the same in both prothoracic and ventral organs, although the bioluminescence of the latter is definitely more orange. No tests for a flavin have been made, but it is very possible that the yellow fluorescence is due to one of this group of compounds.

Physical Properties. The intensity of *Pyrophorus* light has been studied by Dubois (1886) who found $\frac{1}{150}$ of a "Phoenix" candle of 8 to the pound (about $\frac{1}{150}$ candle) while Langley and Very (1890) obtained $\frac{1}{1600}$ candle. The light of *Pyrophorus* in Jamaica, British West Indies, was estimated by Pickering (1916) to be about like that of the first magnitude star, Canopus, or 0.004 candle.

Geipel (1914) found equal illumination on a milk glass screen when *Pyrophorus noctilucus* light was 6 mm and an "Osramlampe" of 0.0034 Hefner unit was 28 mm away. He calculated the "Beleuchtungsstärke" of the pyrothoracic light organ to be 0.00016 "Meterkerzen."²⁶ The glow-worm, *Lampyrus splendidula*, gave half this value.

The latest work is by Harvey and Stevens (1928), who found the brightness of a prothoracic organ of a Cuban *Pyrophorus* sp. (an irregular ellipse 1.5 x 1.2 mm) to be 45 millilamberts (0.045 lumen per cm²) or 0.0002 candle for one organ, about one-tenth of Pickering's value.

The first observation on the spectrum of *Pyrophorus* was made by the great Louis Pasteur (1864), who noted that it was continuous, without bright or dark lines. Later Aubert and Dubois (1884) and Dubois (1886) studied the spectrum in more detail and some of the properties of the light. They noted that the light was not polarized, that it would affect a photographic plate, cause fluorescence of eosin and uranium nitrate but not of quinine or aesculin and would not cause the development of chlorophyll in etiolated plants. The frontispiece of "*Les elaterides lumineux*" is a bust of Claude Bernard, photographed by animal light. Dubois found that the spectrum of the prothoracic organs extended from about 687 to 486 m μ , with maximum emission at 528 m μ .

²⁶ This is a unit of illumination, not of intensity.

Langley and Very (1890) placed the limits of the prothoracic organ light of *Pyrophorus noctilucus* from Cuba at 640 to 468 $m\mu$, with a maximum at 530 $m\mu$. The abdominal organ extended from 663 to 463 $m\mu$. Buck (1937, 41) found in a Jamaican species, *P. plagiophthalmus*, 650 to 505 $m\mu$ for the prothoracic light and 645 to 540 $m\mu$ for the abdominal organ. The difference in color of luminescence of abdominal and prothoracic organs in certain species is real, but as yet the spectral distribution curves have not been worked out. In the Puerto Rican species, *P. luminosus*, the color difference of prothoracic and ventral organs is not marked.

Pyrophorus has also served as the outstanding example of light production without heat. Dubois (1886) pointed out the value of its light as an illuminant since it consisted entirely of visible rays and lacked thermal and actinic rays. He attempted to detect the emission of radiant heat by means of a sensitive thermopile and galvanometer but without certain success. Only a very small deflection of his galvanometer resulted when the insect luminesced.

Langley and Very (1890) also investigated the problem, using a sensitive bolometer. They point out first of all that the total radiation from the most powerful luminous organ (the abdominal one) of *Pyrophorus* which affected their bolometer slightly, would, in the same time (10 seconds), be sufficient to raise the temperature of an ordinary mercurial thermometer having a bulb 1 cm in diameter by rather less than 2.3×10^{-4} C. The radiation from *Pyrophorus* which affected their bolometer was shown to be due merely to the "body heat" of the insect, and it is largely cut off by a plate of glass which is opaque to all wave lengths of 3 μ or more. These waves are given off by bodies at temperatures below 50 C and belong to quite another spectral region than that in which the invisible heat associated with light mainly appears. Langley and Very then compared the radiation from a non-luminous bunsen flame and the *Pyrophorus* light, interposing a plate of glass in each case to cut off the waves longer than 3 μ , and found several hundred times more radiation in the case of the bunsen burner but, nevertheless, perceptible radiation from *Pyrophorus*. The former consisted of radiant heat shorter than $\lambda = 3 \mu$ and extending up to the visible light rays ($\lambda = 0.7 \mu$, since the bunsen flame emitted no light). The very slight effect of the *Pyrophorus* radiation must be due to wave lengths between $\lambda = 3 \mu$ and $\lambda = 0.468 \mu$, the limit of the *Pyrophorus* spectrum in the blue. Langley and Very assumed it to be due entirely to the band of visible light, $\lambda = 0.640 \mu$ to $\lambda = 0.468 \mu$, and assumed that no invisible heat rays were produced. All the energy of *Pyrophorus* light would therefore lie in the visible region and its efficiency

(light rays \div heat + light + actinic rays) would be 100%. In 1902, Langley and Fowle (1908) reinvestigated the radiation of *Pyrophorus* and could detect no heating whatever with the bolometer. "A portion of the flame of a standard sperm candle, equal in area to the bright part of the insects, gave under the same circumstances, a bolometric effect of such magnitude that had the heat of the insect been 1/80,000 as great as that from the candle, it would certainly have been recognized." Thus the search was started for "The cheapest form of light" as Langley and Very's paper was entitled.

CHAPTER XIV

Echinodermata

CLASSIFICATION

This great phylum of almost radially symmetrical animals, exclusively marine, was once grouped with the coelenterates as the Radiata. It contains animals so abundant, so striking in form and so characteristic of the sea that they have common names indicating their marine origin. All systematists agree that the echinoderms should be divided into five classes: (1) Crinoidea (Sea lilies or feather stars), (2) Asteroidea (Sea stars or star-fish), (3) Ophiuroidea (Brittle-stars or snake stars), (4) Echinoidea (Sea urchins), and (5) Holothuroidea (Sea cucumbers). The echinoderms contain about 5,000 living species, in 14 orders and 152 families. The Ophiuroidea contain luminous species and possibly the Asteroidea but none of the other classes.

CASES OF DOUBTFUL LUMINOSITY

Considerable discussion has ensued concerning the possible luminescence of one of the star-fish, *Brisinga endecacnemos*. One of the sea urchins also, *Diadema (Centrochinus) setosum*, has been called luminous by Doderlein (1885), but in this case the report was undoubtedly based on the reflection of light from iridescent spots on genital and interambulacral plates. Many competent observers have failed to detect any luminescence of *Diadema* in complete darkness.

The case of *Brisingia* is somewhat different, as this genus, although belonging to the Asteroidea, is more or less intermediate between star-fish and brittle stars. Asbjørnsen (1856) first dredged *Brisinga endecacnemos* in Hardanger fjord, Norway, at 100 to 200 fathoms, and his statement regarding its luminescence is perfectly definite. Sars and Ludwig studied the genus, but never actually tested the luminescence.

A very careful anatomical and histological study of *Brisinga coronata*, found in the Mediterranean, has been made by Thurst (1916), with special reference to the glandular structure. Like Ludwig, he

had no opportunity to test the animal for luminescence, but in histological sections he found gland cells similar to those in luminous brittle stars, as well as mucous cells. Thurst also studied two species of non-luminous star-fish, *Astropecten aurantiacus* and *Echinaster sepositus*, and found their gland cells to be quite different from those of *Brisinga*. Hence he predicted from analogy alone that *Brisingia coronata* must be luminous. The prediction has not yet been verified, but even though this species is found to be non-luminous, the Norwegian *B. endecacnemus* may produce light, as originally reported.

The Brisingidae include a number of other genera, *Brisingella*, *Odinia*, *Colpaster*, *Freyella*, and *Belgicella*, of which *Odinia elegans* and *Freyella* have been called luminous by Gadeau de Kerville (1890, p.



FIG. 158. An ophiurian by day (left), after H. L. Clark, and the appearance at night, after Dahlgren.

65). They were caught off the west coast of Africa at a depth of 800 to 1,500 meters by members of the exploring ships *Travailleur* and *Talisman* in 1880-83. However, the author has been unable to find any very definite statement on light production of these forms. Perrier (1886) in his book, *Les explorations sous marines*, giving the results of the expedition, merely wrote that *Freyella* "repandent une odeur phosphorée toute particulière; il est fort probable qu'elles sont lumineuses dans l'obscurité." He said that *Odinia elegans* "est remarquable par son apparence délicate," but nothing on its luminescence.

OPHIUROIDEA

There is no doubt of true luminescence among the Ophiuroidea, containing the brittle stars or snake stars, shown in Fig. 158. Possibly the first observer of ophiuroid light was Adanson in 1756. During a voyage to Senegal, he kept what he called sea stars and other animals

in tubs of water in his room and saw them luminesce, but it is quite possible that Adanson's animals, including the sea stars, were dead and luminous bacteria were growing on them. A more certain record of luminous brittle stars is that of Viviani (1805) who published a figure of *Asterias noctiluca*, identified by Panceri (1875) as *Amphiura squamata*. Since then many zoologists—Peron (1807), Tilosius (1819), Quatrefages (1843), Grube (1864), Thomson (1873), Panceri (1875), Lo Bianco (1899), Molisch (1904), and many recent workers—have observed ophiuroid luminescence. A classification of ophiuroids by L. Cuenot with luminous genera in italics is as follows:

Ophiuroidea¹

Ophiuræ

Ophiomyxidae (*Ophiomyxa*, *Ophioscolex*, *Ophiostiba*, *Astrogymnotes*, *Ophiobursa*, *Ophiocanops*, *Hemicuryale*, *Sigsbeia*, *Ophiochondrus*)

Ophiodermatidae (*Ophioderma*, *Pectinura*, *Ophioconis*)

Ophioleptidae (*Ophiura*, *Ophiocten*, *Aspidura*, *Ophionotus*, *Stegophiura*, *Ophiotypa*, *Ophiopyrgus*, *Ophiomastus*, *Ophiomusium*, *Ophiopyrgus*, *Astrophphiura*, *Ophiophycis*)

Amphiuridae (*Amphiura*, *Amphipholis*, *Ophiopus*, *Acrocrida*, *Nannophiura*, *Ophionereis*, *Amphilycus*, *Ophiosphaera*, *Ophiodaphna*)

Ophiactidae (*Ophiactis*, *Ophiopholis*, *Geocoma*)

Amphileptidae (*Amphilepis*)

Ophiocomidae (*Ophiocoma*, *Ophiomastix*, *Ophiarthrum*, *Ophiopsila*, *Ophiocomina*)

Ophiohelidae (*Ophiohelus*, *Ophiolithia*)

Ophiacanthidae (*Ophiacantha*, *Ophiosciasma*, *Ophioplinthaca*, *Ophiomutella*, *Ophiothamnus*)

Ophiothricidae (*Ophiothrix*, *Ophiopteron*, *Ophiopsammium*, *Ophiophthirus*, *Ophiomaza*, *Ophiothela*, *Ophioterresis*, *Ophiogymna*)

Euryalæ

Asteronychidae (*Asteronyx*)

Astroschematidae (*Astroschema*, *Astrobrachion*, *Ophiocreas*, *Astrocharis*)

Trichasteridae (*Trichaster*, *Astroceras*, *Asteromorpha*)

Gorgonocephalidae (*Gorgonocephalus*, *Astrophyton*, *Astrospartus*, *Astrothamnus*)

Biology

Quatrefages (1843) described yellowish green points of light on the arms of small ophiuroids living on algae in the Mediterranean near Chausey. Under a magnification of 30 diameters the points became stripes, presumably the luminous ducts of unicellular glands. Quatrefages came to the conclusion that "motor muscles of the feet are the exclusive source of the light" which he said appeared in a completely closed cavity in a liquid and that luminescence coincided with contrac-

¹ From *Traité de Zoologie* ed. by P. Grassé. Echinodermes by L. Cuenot Vol. XI, 1948.

tion and disappeared when contraction has ceased. The luminous strips above mentioned ran parallel to muscle fibers and the light process was quite "independent of all secretion properly so called."

Panceri (1878) described the luminescence of *Amphiura squamata*, common in the Gulf of Naples, in a one-page appendix to his monograph, "La luce e gli organi luminosi di alcuni annelidi." His figures indicate that only the arms are luminous, the light coming from two oval luminous dots on each segment, near the points where the tube feet arise. When stimulated, the ophiurans gave repeated flashes of light and then scurried quickly away without lighting. Consequently Panceri was inclined to doubt the validity of Quatrefages' alleged parallelism between muscle contraction and luminescence, especially as Peron² had described an *Ophiura phosphorea* from the island of Bernier, which luminesced not only on the arms but also on the disc where there were no muscles. Panceri hoped to observe young ophiurians, whose tissues would not be so opaque, but his untimely death in 1877 put an end to his luminescence studies.

It is a curious coincidence that during the twentieth century, luminescence of ophiurians should have been intensively studied by five different men in the years 1907-9, Mangold (1907, 08) of Greifswald, Sterzinger (1907) of Innsbruck, Reichensperger (1908) of Bonn, Trojan (1908, 09) of Prague, and Sokolow (1909) of St. Petersburg. The work has been in most cases histological, directed to identifying the photogenic cells in the luminous areas and the method of lighting, but Mangold, in addition to histological studies, has carried out a number of physiological experiments. Four of the workers regarded the luminescence as intracellular while Sterzinger considered that an extracellular luminous slime is produced. Kato (1947) has described intracellular luminescence from eosinophil cells in *Amphiura kandai*.

The luminescence of ophiuroids may appear from different regions depending on the species. Grimpe (1930) has summarized the facts for Mediterranean species in a convenient table. In the commonest form, *Amphipholis* (*Amphiura* or *Ophiolepis*) *squamata*, both Mangold and Reichensperger have described the light as coming from the proximal part of the spine basal plates with two lighting fields in each joint, but Sterzinger disagreed with this, holding that the light came from the feet.

In *Amphiura filiformis*, Mangold noticed light from the spines only. In *Ophiopsila annulosa*, Mangold and Reichensperger described light from the spines, including the vibratory spines, the ventral plate and the sides of the lateral plates next to the disc, while in *Ophiopsila*

² Quoted from Panceri (1878).

aranaea the same regions as in *P. annulosa* were luminous except the spines.

In *Ophiacantha bidentata* Sokolow described the light from spines, basal and lateral plates and in *Ophiacantha spinulosa*, often regarded a synonym of *O. bidentata*, Trojan described light from the ambulacral feet. There is no doubt but that, depending on the species, either plates or spines may be luminescent, but there is doubt regarding the feet.

As in the case of so many other luminous groups a closely allied species, for example, *Amphiura chiajei*, produces no light, while *A. squamata* is brightly luminous. According to Mangold (1907) *Ophiothrix fragilis*, *Ophioderma longicauda*, *Ophioglypha lacertosa* and *Ophiomyxa pentagona* do not luminesce, but living young of *Amphipholis squamata*, while still in the mother and less than 2 mm long are luminous. Their light can be seen shining through the disc.

Histology

Mangold (1910) has given a good summary of the histology, based on his own researches as well as those of Sterzinger, Trojan, Reichen-sperger, and Sokolow. Without discussing the details of structure in different ophiuroids or all the points of disagreement among workers as to the facts, it may be said that the majority agree that in most species the photocytes are unicellular gland cells or groups of gland cells in the connective tissue under the plates. This gland cell, which is rather large and filled with granular and mucous material, staining in thionin and mucicarmine, sends a long projection into the epithelium. There appear to be small reservoirs along this duct and a pore that penetrates the cuticle. In some cases nerve fibers can be recognized going to these cells which are absent in non-luminous regions and in non-luminous ophiuroids. The luminous cells and ducts are shown in black in Fig. 159.

Despite the ducts, the luminescence has been considered intracellular or intraglandular and appears to be similar to that in polynoid worms (Kutschera, 1909). The fact that it is not possible to separate a luminous secretion from the animal even by squeezing the luminous regions, that the light is closely connected with the life of the animal, and that the contours of the lighting parts remain constant all speak for intracellular luminescence. Most workers have assumed that light must appear within the cell and that the duct remove only waste material, or that the light occurs at the opening of the duct. Sokolow called the light intracellular but did claim that in *Ophiocantha bidentata* it was connected with a fluid substance because he observed luminescence

spreading from luminous spots on the arms when this species was placed in fresh water.

Sterzinger alone has described extracellular luminescence of *Amphiura squamata* from "gland cells in the outer epithelium of the feet-tips (Spitze), which secrete a mucus collecting in intracellular spaces and thrown out of openings in small papillae at the front ends of the feet." She emphasized the fact that *Amphiura squamata* also produces a non-luminous slime from the feet, and non-luminous ophiuroids secrete a

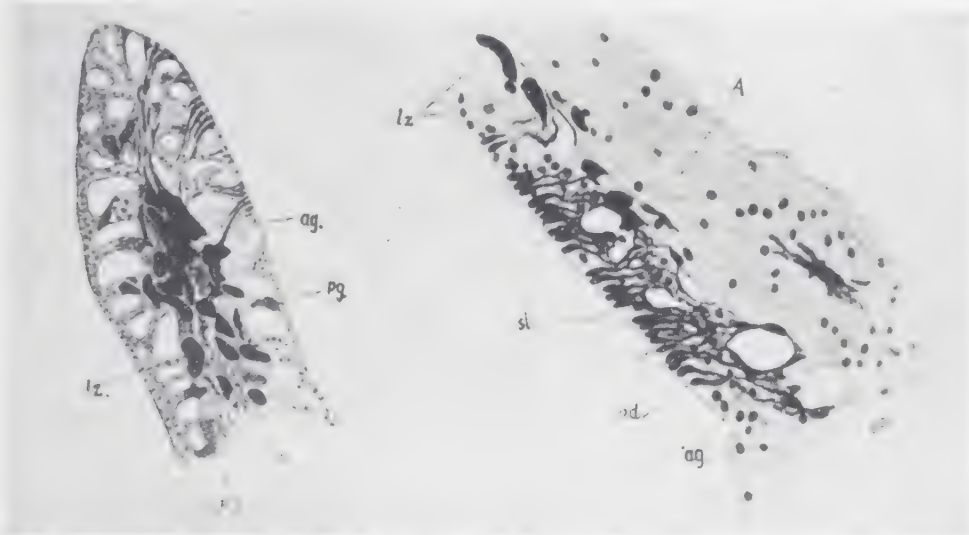


FIG. 159. Left. Section of a spine of *Ophiopsila annulosa* showing photogenic cells and ducts stained black; lz, light cells; ag, ducts, pg, enlarged reservoirs on distal part of duct. Right. Lateral spine of *Amphiura filiformis*, with many ducts. After Reichensperger.

slime which aids in clinging to vertical walls. However, it is difficult to reconcile this view with the findings of others.

Physiology

That the greenish light of Ophiurians is not spontaneous but appears on stimulation has been observed by many workers. Sokolow (1909) thought the luminescence was independent of the central nervous system because broken arms and spines will light, but actually the normal light emission is undoubtedly a nerve reflex. He was particularly impressed by the brilliant greenish yellow light when the animal was placed in fresh water.

The luminous response of a vigorous animal to mechanical stimulation is usually described as a "Schimmer," like lightening. Mangold (1907) regarded ophiurans as difficult to stimulate mechanically. They readily fatigued, but after exhaustion from mechanical or elec-

trical stimulation were still capable of lighting if dropped into sublimate alcohol fixing fluid. Stimulation of one part of an arm sets off an impulse which moves along the arm in either direction as a light wave and will pass by way of the nerve ring in the disc to other arms. The impulse must be carried by radial nerves on the under side of the arm, because, if the nerve is cut the impulse is unable to pass the cut region. Mangold noticed that isolated spines of *Ophiopsila annulosa* will luminesce in strong NaCl solution, but was not certain whether the stimulus was a direct one or took place through nerve fibers.

Many other chemicals, acetic acid, ammonia, alcohol, distilled water, etc., act as stimuli for continuous luminescence and are useful in detecting the locus of light emission. Mangold studied the effect of a number of alkaloids, usually in 1% solution in sea water. Cocaine, muscarine and pilocarpine were strong stimulants to luminescence, but atropine did not excite and appeared to have an inhibitory action on the utilization of the luminous material. The inhibition could be overcome by strong mechanical stimulation. He did not determine whether in more concentrated atropine the inhibition would be complete or whether the well-known antagonism between atropine and pilocarpine occurred. Chloroform produced a long-lasting light, and 25% chloral hydrate in sea water at first stimulated and then narcotized.

Biochemistry

Practically no work has been carried out on the chemistry of luminescence. Mangold (1907) observed and the author has confirmed the fact that light material cannot be easily conserved by drying the luminous parts. No light appeared when the dry organ was again moistened.

The author (1926) determined that oxygen is necessary for luminescence of *Amphiura squamata* and that the luminescence of the whole animal is not inhibited by sunlight. When an *Amphiura* is ground in a mortar with sea water, a bright yellowish light appears which does not last long. The addition of cytolytic agents (saponin, Na glycocholate, chloroform, or distilled water) to this sea water extract excites no luminescence. It is also not possible to demonstrate a luciferin-luciferase reaction, even when the *Amphiura* luciferin solution is prepared in absence of oxygen. The sea water extracts of *Amphiura* which should contain luciferase give no light with *Cypridina* luciferin nor does *Cypridina* luciferase light when extracts of *Amphiura*, which should contain luciferin, are added.

In ultraviolet light both *Amphiura squamata* and *Ophiopsila annulosa* are brightly yellowish green fluorescent. The effect is observed pat-

ticularly after they have been stimulated to luminesce strongly and the bioluminescence has disappeared, possibly indicating that an oxidation product of the luminous reaction is involved. The fluorescence is very marked and bright and in *A. squamata* can be observed under the microscope as a network of yellowish green fluorescent material on the plates. The ability to fluoresce lasts for a long time and is very different from the bluish fluorescence of the skeletal parts. In *Ophiopsila aranea* the fluorescence appears as bright points of yellow light evenly distributed over the plates and the same yellow fluorescent particles are present on the feet.

CHAPTER XV

Tunicata or Urochordata

CLASSIFICATION

As the name indicates, this subphylum consists of globular or cylindrical animals surrounded by a cellulose-like covering, the test or tunic. Although adults have the appearance of invertebrates, the larval development discloses a notochord and definite relations with the Vertebrata. Some 1,300 species have been described. Most of these belong to the Ascidiacea and are plant-like, sessile organisms, with opaque tests, unlikely to contain luminous forms. The Larvacea and Thaliacea are transparent and pelagic, and many can produce light. Classification of the Tunicates by H. Lohmann, G. Neumann, and J. Huus is given in the following table, with the luminous genera in italics.

Tunicata

Copelata or Perennicordata

Larvacea or *Appendiculariae*

Oikopleuridae (*Oikopleura*, *Folia*, *Megalocerus*, *Stegosoma*, *Chunopleura*,

Pegalopleura, *Althoffia*, *Bathochordaeus*)

?Fritillaridae (*?Fritillaria*, *Tectillaria*, *?Appendicularia*, *Kowalevskia*)

Acopa or Caducicordata

Thaliacea

Pyrosomida

Pyrosomatidae (*Pyrosoma*)

Cyclomyaria or *Multistigmatea* or *Doliolida*

Doliolidae (*Doliopsis*, *Doliolum*)

Desmomyaria or *Astigmatea* or *Salpida*

Salpidae (*Cyclosalpa*, *Brooksia*, *Ihleia*, *Salpa*, *Ritteriella* (*Ritteria*)

Apsteinia, *Metcalfina*, *Thetys*, *Pegea*, *Traustedia*, *Thalia*, *Iasis*)

Octacnemidae (*Octacnemus*, *Polyoctacnemus*) deep sea

Ascidiacea

Enterogona

Polyclinidae (14 genera)

Clavelinidae (15 genera)

Didemnidae (7 genera)

?Cionidae (5 genera, including *?Ciona*)

Agnesiidae (2 genera)

Corellidae (6 genera)

Asciidiidae (10 genera)

Pleurogona

?*Styelidae* (27 genera, including ?*Botryllus*)

Pyuridae (9 genera)

Molgulidae (10 genera)

Hexacrobylidae (2 genera)

LARVACEA

As contrasted with doubtful luminescence in the Ascidacea, there is no question of the luminescence of these tiny free-swimming and rather aberrant forms, the Appendiculariae, whose tail contains a notochord which persists throughout life. The tunic is large, devoid of cellulose and shed periodically, and the animal can move about inside as in a house.

Found in all seas, the Appendiculariae are most abundant well away from land. One of them (*Appendicularia*) is included in Giglioli's (1870) list of luminous forms, observed on a voyage around the world in 1865-68. Lohmann (1899) was the first to observe carefully the luminescence of *Oikopleura albicans* in Messina, and a detailed account by him (1933) will be found in the Kückenthal-Krumbach "Lehrbuch der Zoologie." In this species there are three different methods of luminescence at different stages of development. First, in the free-swimming tunic-less form, Lohmann observed a bright greenish flashing on stimulation, of short duration, that came only from the trunk, not from the tail. The exact location within the trunk was not certain. Second, at the time the tunic unfolded, the light of the whole trunk with the exception of the gonadal cavity became brighter and white and was continuous. The luminescence came from the oikoplast epithelium, that secretes the tunic. Third, after the tunic had unfolded and the animal had rested, the continuous light disappeared and flashes of light again occurred on stimulation. Lohmann was not certain what substance produced the light in this stage but believed it to be the oikoplast epithelium and the secretion of mouth glands.

These mouth glands secrete a fluid that covers the apex and under surface of the beak and forms at the base of the tail chamber a symmetrical pattern of bands or points. Such a secretion is found in all appendicularians and is fluorescent, appearing orange by reflected light and green by transmitted light. In *Fritillaria pellucida* its color is orange and Lohmann suggested that all appendicularians may be luminous. The small size of these forms—*Oikopleura flabellum* of the Atlantic and Pacific oceans attains a maximum length of 12 mm. of

which 10 mm is tail—makes investigation difficult, but there can be no doubt of their self-luminosity. A possible relation to luminous bacteria has not been considered.

THALIACEA

In this group, the salpas and the pyrosomas, pelagic transparent tunicates, luminescence is rather widespread. The best-known form, *Pyrosoma*, literally “fire-body,” shown in Fig. 160, is a floating colonial tunicate of tropic seas and has stimulated some of the most enthusiastic and striking descriptions of marine phosphorescence. *Pyrosoma* has



FIG. 160. Colonies of *Pyrosoma*. Photo by the author.

been called a “splendid spectacle” and its luminescence has been described as “romantic, imposing, and majestic.” It has been compared to “red glowing iron” and is said to have been present in such numbers as to illuminate the sails of ships at night and to render objects in the cabin easily visible (Bennett, 1833, 37).

Although not as striking as *Pyrosoma*, the genus, *Salpa*, and its numerous subgenera, and the various species of *Doliolum* are also almost universally luminous, while the deep sea form, *Octacnemus*, may be luminous, although nothing is known for certain. The *Salpae* pass through what is frequently called an alternation of generations, a solitary form alternating with an aggregated one, which constitutes a “*Salpa* chain.” Luminous organs may be present in both generations or lacking in one of them or in both.

Pyrosoma

This single genus, *Pyrosoma*, contains about ten species and several subspecies, undoubtedly the most extraordinary colonies of luminous animals in the living world. It is abundant as plankton of warm seas, ranging from the surface to depths of 200 meters. The colonies may attain a length of 4 meters, although they are usually 3 to 10 cm, while individual animals, usually 4 to 5 mm, are sometimes 20 mm in length. Although Lesueur (1815) recognized the similarity of *Pyrosoma* to ascidians, and Huxley (1851, 60) gave a detailed account of its structure, the exact position of *Pyrosoma* among tunicates is somewhat doubtful. Some authors place the animal with the Thaliacea, others with the Ascidiacea.

Pyrosoma was "discovered" by Peron (1804) in December, 1800, during an expedition to Australia and New Zealand. It has excited the wonder and admiration of all voyagers since that time. Bory de St. Vincent (1804) described it as *Monophora noctiluca*. Kuhl (1822), Bennett (1833, 37), Meyen (1834), Vogt (1848), Huxley (1851), and naturalists on the *Challenger* (Moseley, 1879) and other deep sea expeditions have recorded its brilliant luminescence. Bennett (1833) noted that *Pyrosoma* luminesced only on stimulation and thought that the light-emitting power resided in brown or red specks in the animal, which, however, "when removed from the body did not emit light." In 1837, after another voyage in which he again observed *Pyrosoma atlanticum* in tropical seas, Bennett referred to the light as coming from "small brown particles" embedded in the body. Contrary to the case of luminous medusae, no luminous slime was observed but "if the *Pyrosoma* be cut open and immersed in water, the brown particles that escape diffuse themselves through the fluid, and shine as numerous scintillations, independent of the perfect structure." Actually, the pigment bodies have nothing to do with the luminescence, which many later observers have shown to be restricted to the paired luminous organs, the eggs in the ovary, and the embryos in the cloaca.

Bennett also noted that "touching one small portion of the body (colony) is sufficient to produce a brilliant glow throughout the whole" and that fresh water is "a powerful and permanent stimulus on marine Noctilucae." The *Pyrosomae* in fresh water "never ceased glowing with their brightest refulgence until life was extinct, which was not until after the lapse of many hours."

The genus *Pyrosoma*, all species of which are luminous, is made up of two sections, which Metcalf (1919) considered sufficiently different to be separate genera. Each zooid of the section of *Pyrosomata fixata*

possesses, among other characteristics, four luminous organs, while the *Pyrosomata ambulata* possess only two. Both sections of *Pyrosoma* are hermaphrodite. Some species of the ambulata are protogynous and form relatively small colonies, less than 5 cm long, while others are protandrous and form large colonies, up to 100 cm in length. A single animal is shown in Fig. 161.

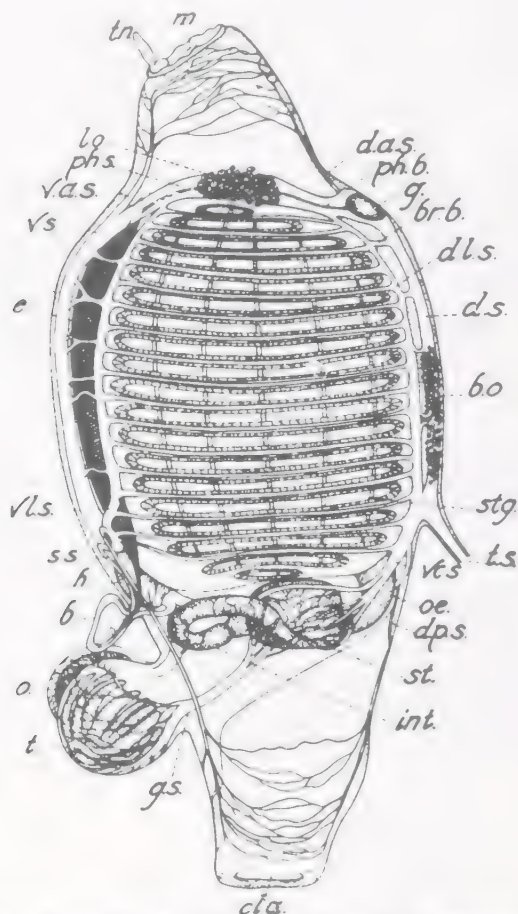


FIG. 161. A single animal of *Pyrosoma atlanticum*. The light organs (lo) can be seen above the branchial bars (br.b.). After Metcalf.

Histology and Embryology. All species of *Pyrosoma* possess at least two slender elliptical or circular masses of cells at the forward end of the branchial sac of each zooid between the inner and outer tunic and attached to the latter. Lesueur and Savigny thought these cells were ovaries. Huxley considered them kidneys, and Joliet called them glands. It remained for Panceri (1872, 73) to discover that they were light organs and to give the first good account of *Pyrosoma* luminescence, pointing out the errors of Bennett (1833, 37) and Meyen (1834).

who thought the red pigment cells covering the wall of the esophagus and stomach were luminous. Panceri described the organs as made up of yellowish¹ cells, containing photogenic fat droplets and no nucleus, and believed them to be derived from the external layer of the blastoderm.

According to Julin (1909, 12) the anterior luminous cells (of *P. giganteum*) arise from the test cells, called inner follicle cells by Kowalevsky and calymnocytes by Salensky. They do not contribute to the formation of the embryo but act as nurse cells and also secrete the tunic or test of *Pyrosoma*. Those test cells lying in the periphar-

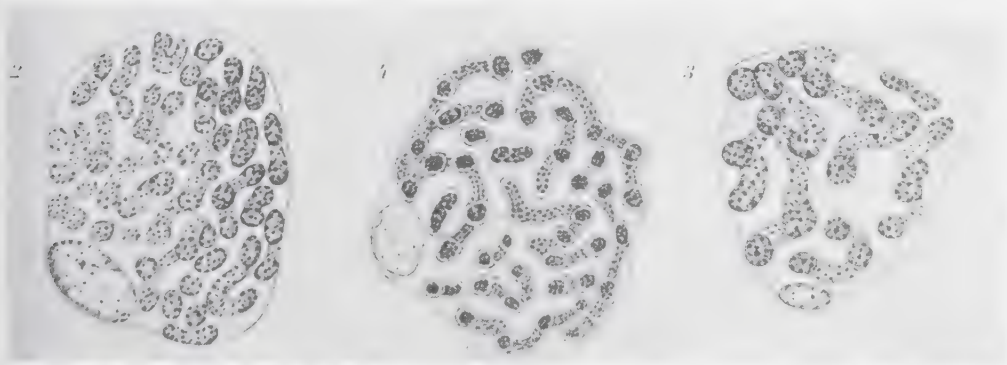


FIG. 162. Bacteria-like bodies in the luminous cells of *Pyrosoma*. After Julin.

ngeal blood space become permanently modified to form the luminous organ. The luminous cells contain a nucleus and also curved cytoplasmic inclusions which are not fatty in nature, contrary to the belief of Panceri, for neither Julin (1912) nor Burghause (1914) could detect fat.

During segmentation and younger embryonic stages all the test cells, including those of the egg, are luminous, giving rise to the luminescence of embryos, but test cells not destined to form the light organ later lose their ability to light and function in forming the test or tunic. Julin (1912, 13) described the inclusions of the test cells as consisting of a closely convoluted tube whose walls and inner reticulum are achromophilic, but at the knots of the reticulum are to be found numerous powdery granulations of nuclein.² They are shown in fig. 162.

In 1914, Pierantoni published his theory that the light of the firefly was due to symbiotic luminous bacteria, and Buchner (1914) suggested a similar origin for *Pyrosoma* light, based on the investigations of Julin. Later Pierantoni (1921), in a careful study of those photo-

¹ E. B. Harvey was unable to detect a yellow color in the photogenic cells of a *Pyrosoma* examined at Monaco in January, 1921.

genic cells, also decided that the inclusions were actually modified luminous bacteria. Like Julin he described them as irregular, curved, tubular bodies, 2 to 3 μ thick and 10 to 30 μ long, with an internal network in whose meshes strongly staining granules might be seen. There were also oval spore-like structures of chromatin, usually at the ends of some of the curved inclusions. These spores he described as becoming free from the bacteria (which then degenerate) and passing into the pharyngeal blood sinus. They finally reach the genital sinus where they infect the eggs during the last stages of their growth, passing into the follicle cells, which divide amitotically and become test cells. These test cells then migrate between the blastomeres into the developing embryo and the spores develop into the curved luminous bacteria. The test cells arrange themselves in a ring-shaped zone of forty to fifty cells at the margin of the germinal disc. When the four primary ascidiozooids arise from the cyathozooid, the test cell ring is broken and the test cells pass into the circulation and later build the four pairs of light organs of the primary ascidiozooids.

In the formation of secondary ascidiozooids the test cells of the primary ascidiozooids do not divide but degenerate, and the bacterial bodies become free in the peripharyngeal blood sinus. They are taken up by mesenchyme cells which themselves come from the dorsal blood forming organ and which wander through the stolon into the bud of the developing ascidiozooid, there to form the new light organ, thus repeating the cycle. An excellent account of the histology, embryology and physiology of luminescence in *Pyrosoma* will be found in Buchner's book (1921) and in Neumann's (1934) paper.

Physiology. Although Huxley (1851), Moseley (1879), and others have described the wave of light in *Pyrosoma*, Panceri (1872, 73) must be considered the first worker to carry out definite physiological experiments. He noted that the luminescence was excited locally by various stimuli (shock, touch, rubbing, alcohol, and ether) and then spread to other zooids. He observed a similar propagation in *Pennatula* and compared the two, finding that in *Pyrosoma* the velocity of propagation was less, the light less brilliant, and there was no repetition of the impulse after a single stimulation. Panceri described smooth muscle ribbons connecting one zooid with another, a set parallel to the long axis of the colony and another at right angles to it, and thought that nerves might accompany the muscle system and carry the impulses that excited luminescence.

Panceri tried to stimulate *Pyrosoma* with electric currents without success, but Polimanti (1911) found no difficulty in stimulating with induced shocks and also observed that the colony was particularly

sensitive to mechanical disturbance, since bubbles of air rising against a zooid or a slight jar of the aquarium were sufficient to stimulate. According to both Panceri and Polimanti, a *Pyrosoma* colony will occasionally light spontaneously when no external stimulus can be observed. Probably some small organism living among the zooids has, by its movement, irritated the animal. All observers have noted that after continued stimulation the luminescence diminishes greatly through fatigue.

The most extended physiological investigations have been carried out by Polimanti and Burghause (1914), both of whom studied the wave of light which spreads over the colony from a stimulated region. Polimanti found that this wave may sometimes skip individual zooids, appearing at the end opposite the stimulated region without affecting the middle. There is great variation in irritability, depending on the condition of the colony. The longer the colony is kept in the laboratory the stronger must be the stimulus in order to obtain luminescence.

Polimanti discovered the response of *Pyrosoma* to light. One of the best methods of excitation came from the turning on (or turning off) an electric lamp. The light stimulated the zooids to luminesce, either in a local spot or over the whole colony. Polimanti also reported that if two *Pyrosoma* colonies were in the same dish of sea water and one colony luminesced, the second colony would not be excited by the light of the first, but this statement has been contradicted by Burghause, who made many experiments to show the response of neighboring colonies to the light of each other. Polimanti's animals must have been in poor condition.

Burghause showed that the response of one *Pyrosoma* to the light of another in the same dish could not have been due to mechanical stimulation. When the animals were placed in sea water in separate dishes close to each other, the luminescence of one stimulated the other. Moreover, the reflex luminescence of *Pyrosoma* could be elicited by the luminescence of ctenophores, siphonophores, and the fire-fly, *Lampyris italica*.

Although Panceri had reported no effect of daylight in suppressing luminescence of *Pyrosoma*, Burghause did find such an effect. Mechanical stimulation of colonies kept long in daylight frequently gave no response, and the time for luminescence to appear when ammonia was added was much longer than with colonies that were collected at the same time but had been kept in the dark. Sometimes a *Pyrosoma* would not react to a light stimulus, and Polimanti observed that if the colony was allowed to rest for some time with only occasional mechanical stimulation, it regained the sensitivity to light.

The latent period of luminescence after stimulation was from one to five seconds, and the resulting luminescence lasted five to thirty seconds before disappearing. The latent period was also longer the weaker the stimulus, and a weak stimulus resulted in a weak luminescence, which remained localized, not spreading over the colony.

The propagation of the luminous wave over the colony might come about in three ways: (1) a direct nerve transmission as favored by Panceri; (2) a mechanical effect from contraction of zooids in the stimulated region, with the resultant movement stimulating adjacent zooids to luminesce; (3) a light response reflex, adjacent zooids responding to the luminescence of zooids previously stimulated.

Burghause, in discussing the second possibility, reported that he could observe no muscular movement accompanying the transmission of a light wave over the colony. He did not observe the lighting in scattered regions, which might mean that a transmitted impulse had skipped the intermediate areas as Polimanti had described, but noted only a rapid wave-like spread of light. Polimanti's observation, however, could be explained by a light reflex in which certain zooids are more favorably situated to receive the luminescence of the initial group that start the reflex.

Burghause described an experiment in which a piece of the colony containing several zooids was cut out and then replaced in its original position. When a light wave was started and passed over the colony, the excised zooids lighted at the proper time. This experiment would appear to rule out any sort of nerve transmission of the luminous wave. Burghause, therefore, came to the conclusion that light wave transmission is by reflex luminescence response of each zooid to the luminescence of its neighbor.

Pyrosoma colonies can be narcotized. If alcohol is added drop by drop to sea water containing the colony, the animals are unable to luminesce on stimulation. However, alcohol in greater concentration has a stimulating effect for, when added quickly, luminescence appears and lasts some time, a type of stimulation noted by most previous observers.

The use of the light of *Pyrosoma* is a mooted question. Most workers have thought of it as defensive, but there are records of *Pyrosoma* in the stomach of fishes, and fish have been observed to eat *Pyrosoma* in aquaria.

Biochemistry. Panceri found that if a *Pyrosoma* colony is pressed through a cloth, the resultant extract remains luminous for a time but soon becomes dark. On mixing with fresh water the dark extract again gives off a brilliant light. Panceri also observed that if a colony in sea

water is slowly heated, spontaneous luminescence begins at about 28°C and remains shining until about 60°C.

As with many other luminous organisms, the dried luminous material of *Pyrosoma* will again luminesce when fresh water is added and behaves very much like the luminescent matter of *Pennatulæ*, *Medusæ*, pholads, *Chaetopterus*, and *Beroë*. Panceri wrote: "Neither daylight nor the action of solar rays on *Pyrosoma* lessen the luminous power, as in the case of *Beroës*. The luminous substance is in all probability fatty matter." However, Burghause (1914) tested the light cells of *Pyrosoma* with osmic acid and found no blackening and also no coloration with alkanna or Sudan III and consequently concluded that fat was not present.

Burghause also tested for the presence of luciferin and luciferase, with negative results, and the author (1922) was unable to demonstrate the luciferin-luciferase reaction with a species of *Pyrosoma* at Monaco. Moreover, *Cypridina* luciferin gives no luminescence with extracts of *Pyrosoma* that should (from method of preparation) contain luciferase, nor will *Cypridina* luciferase luminesce with *Pyrosoma* extracts that should contain luciferin.

Polimanti (1911) stated that *Pyrosoma* luminescence was not an oxidation, basing his conclusions on rather absurd grounds, such as the color of the light, on the fact that no heat could be detected and that the luminescence intensity increased very little with increase in temperature. Burghause (1914) came to the same conclusion, since he found that KCN and chloral hydrate failed to suppress the luminescence, as they suppress oxidations of living cells. In fact these two substances evoked a luminescence that often lasted an hour or more.

It is now well recognized that cyanide has little effect on the luminescent reactions of many organisms whose light emission is dependent on oxygen, and that the above evidence is not evidence at all. Actually no one has tested the luminescence of *Pyrosoma* (or *Salpa*) in neutral gases such as nitrogen or hydrogen, and it is not known whether dissolved oxygen is a necessity for luminescence. The experiment should be undertaken at the first opportunity.

The fact that cyanide has no effect on *Pyrosoma* luminescence while it does decrease the light of luminous bacteria, together with the excitation of *Pyrosoma* luminescence by various stimuli and particularly the long-lasting light in distilled water, which extinguishes the light of bacteria immediately, are all contrary to the idea of symbiotic bacterial luminescence in this animal.

Physical Characteristics of the Light. Peron (1804) has described the light of *Pyrosoma atlanticum* as first red, then orange, then greenish, and finally sky blue. Meyen (1834) referred to the light as greenish blue, Vogt (1848) as wine yellow and Moseley (1879) as fire color. Panceri (1872) described the light of *Pyrosoma giganteum* as "azzurrina" and *P. atlanticum* as "policroica"; Polimanti (1911) called the light of *P. elegans* greenish, while Julin (1912) and Burghause (1914) both speak of *P. giganteum* luminescence as greenish blue. Almost every observer has described a different color.

The meaning of this play of colors is somewhat uncertain. Polimanti observed that as the animals became crowded together and started to die, the luminescence became more red, as it also did at a high temperature. Burghause believed that this luminescence color change was due to the fact that the colonies become more opalescent on heating and that the red pigment cells disintegrated and the red pigment diffused through the colony, acting as a red filter, and Polimanti also noted the change in the pigment of *Pyrosoma*. The early observers may have been overenthusiastic in their descriptions of the luminescence tints as well as misled by changes in the color sensitivity of the eye which occur during dark adaptation, but the subject is of great interest and requires further investigation.

A modern spectrographic study is much needed. The only work is that of Secchi (1872), who reported in a short note that he examined with a spectroscope the light of some *Pyrosomae* sent by Panceri and found the spectrum to be continuous but with less red in it than that of the glow-worm (*ver luisant*).

Salpa

It is possible that observation of luminous *Salpae* goes back to Aelian, in the third year A.D., who mentioned a flickering light coming from a sea growth, which Steuer (1911, p. 1) believed might be the luminescence of *Salpa africana maxima* which he observed as great chains in the Mediterranean. In more modern times the luminous organs of *Salpa* were certainly seen by Peter Forskal, friend of Linnæus, who first accurately described *Salpae* in 1775 and proposed the generic name. Forskal did not know that certain clumps of cells in the animal were luminous, and their function has given rise to much speculation, being regarded as ovaries or renal organs. According to Ehrenberg (1834), Chamisso saw luminous *Salpae* in 1819, and Baird (1851) in his article "On the luminousness of the sea" recorded a *Salpa* as emitting light and his figures depict what were evidently *Salpae*. All animals retained their "luminous property for upwards of 12 hours

after they were placed in a tumbler of clear salt water." Salpae have also been noted as luminous by A. Agassiz and many others.

Brooks (1893) first studied the luminous region in *Salpa pinnata* and pointed out the histological similarity to the luminous cells of *Pyrosoma*. He reported that their light could be seen despite the brightness of full daylight and also that "in sunlight the organs have a tinge of purple, but at night their light is as white as the glow of an incandescent wire."

According to Metcalf (1918) the luminous organs have diagnostic value. One of his drawings is reproduced on Fig. 163. In the solitary form of *Cyclosalpa pinnata* they are a series of five-paired spindle-shaped glands between the body muscles, halfway up the sides of the body; in aggregated form, there is only a single long-paired organ between the second and third body muscles. In *Cyclosalpa floridana* the aggregated form lacks luminous organs, but the solitary form has five pairs, as in *C. pinnata*, although the first and fifth pair are only slightly developed or lacking, and the others are smaller. In *Cyclosalpa affinis* no luminous organs are present in either the solitary or aggregated form. Metcalf has published a list of recorded luminous species.

Histology. Julin (1912, 13) has pointed out that the chief difference between the luminous organs of *Cyclosalpa pinnata* and of *Pyrosoma giganteum* has to do with the framework of connecting cells. In the lateral luminous organs of Salpae there is a framework which is lacking in *Pyrosoma*. Within the framework are to be found free luminous blood cells much like the luminous test cells of *Pyrosoma*, except for their smaller size. They contain similar convoluted tube-like inclusions and play the double role of blood formation and luminescence. Julin (1913) remarks that "It would be interesting to ascertain whether the luminous cells of the lateral organs of the solitary form of *Cyclosalpa* are derived from the cells of the test of the egg, as is the case of the cells of the luminous organ of the four ascidiozooids" of *Pyrosoma*. These luminous cells of *Salpa* are shown in Fig. 164.

This proposed investigation has now been carried out by Stier (1938), who made a detailed study of the early embryology of *Cyclosalpa pinnata*, based on fixed material furnished by Buchner. At the twelve-cell stage there appear "plasmafragments" which in all likelihood represent luminous bacteria which have infected the cells. These plasma fragments go through cyclical form changes that are similar to those of undoubted symbionts.

Not all blastomeres contain such apparent symbionts, but they are present in definite ratios. The cells without symbionts divided mitot-

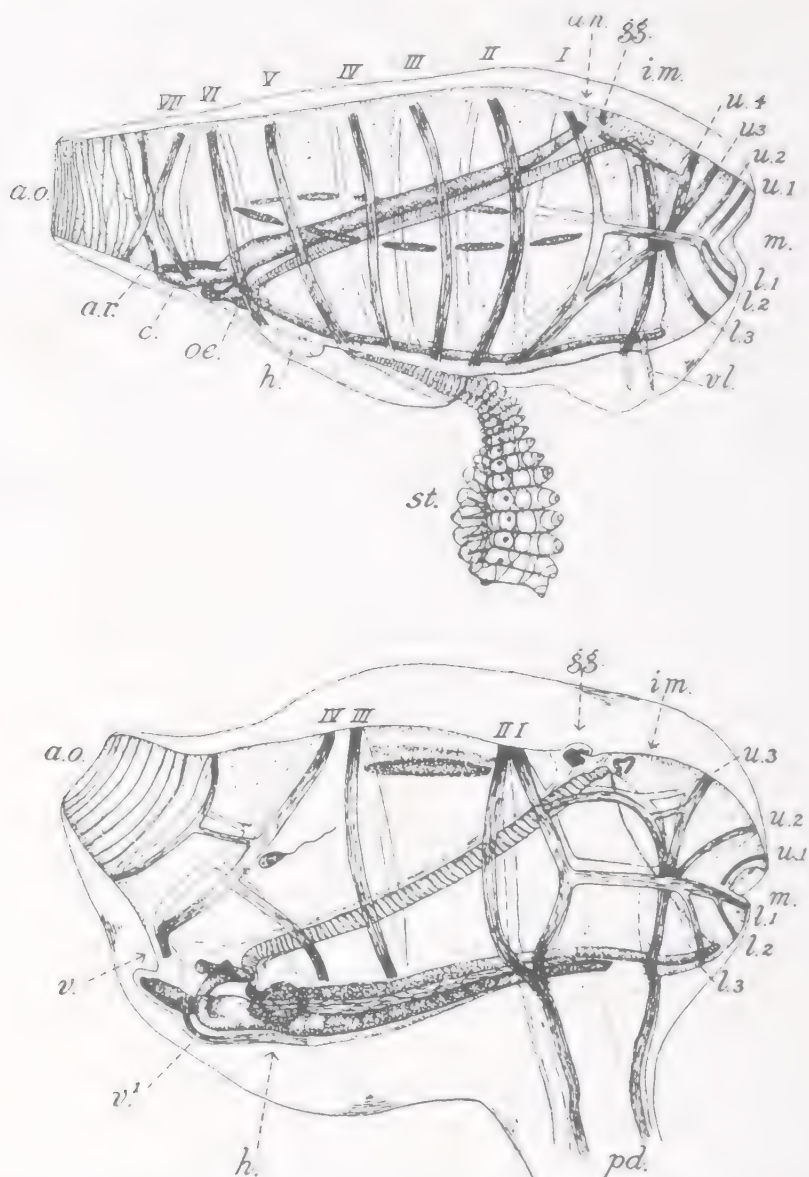


FIG. 163. *Cyclosalpa pinna*. Solitary form above and aggregated form below. The light organs of the solitary form are halfway up on each side of body, a series of five spindle-shaped structures between the body muscles, whereas in the aggregated form the luminous organ is single on each side, in the interval between second (II) and third (III) body muscles. After Metcalf.

ically into symbiont-free daughter cells (which form the embryo) and symbiont-containing cells which later disintegrate. The freed symbionts are then taken up by "kalymmocyten," which presumably form the light organ of the adult. The kalymmocytes of Stier and Salensky have been usually identified with the test cells or inner follicle cells of

Kowalevsky in *Pyrosoma*; thus the luminous organ embryology is very similar in *Salpa* and *Pyrosoma*. Ihle (1935) has given a detailed account. No experiments on the physiology or biochemistry of *Salpa* have been undertaken, but it is probable that the behavior of the luminous cells would be similar to those of *Pyrosoma*, where the evidence is against the presence of luminous bacteria.

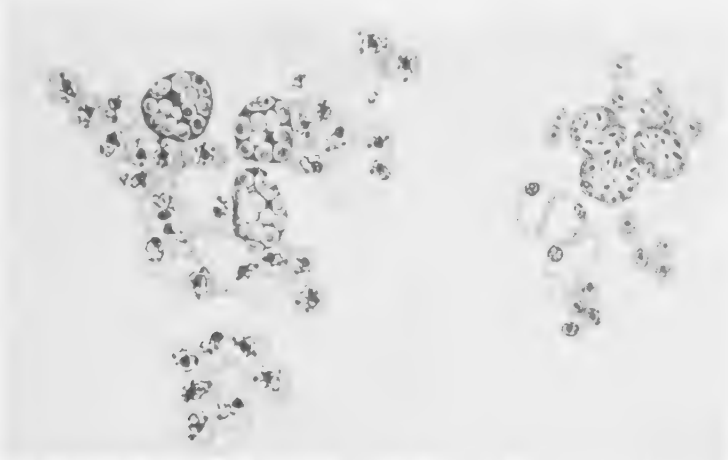


FIG. 164. Enlarged view of luminous cells from the solitary form of *Cyclosalpa pinnata*. After Steier.

ASCIDIACEA

All records of ascidian luminescence are over a hundred years old. Landsborough (1842), whose superficial observations on hydroids and polyzoa have already been mentioned, noted that *Botryllus Schlosseri* was phosphorescent and gave "the overspreading glow of one massy creature which all shown through with a lurid and sullen looking fire."

Will (1844) in a short paper on the light of some animals at Trieste, mentions, among others as luminous, *Phallusia* (*Ciona*) *intestinalis*. No further recent records of these forms or of any other ascidians are to be found in the literature, and the *Ciona intestinalis* which occurs at Woods Hole, Massachusetts, is not luminous. The only explanation of Will's record, apart from a serious error in identification, is a possible infection of the *Ciona* at Trieste with luminous bacteria. Although his infection has not been observed to occur naturally, Skowron (1926) has artificially injected *Ciona* with luminous bacteria at the Naples Aquarium, and the animal remained luminous for two or three weeks. The ovary was particularly bright. Had such an infection occurred naturally the animal would certainly have been classed as luminous. Gadeau de Kerville (1890) has offered the same bacterial explanation of both luminescent *Ciona* and *Botryllus*.

CHAPTER XVI

Pisces

INTRODUCTION

In the great phylum Chordata luminous animals are found only among the more primitive groups. Of the three subphyla, the Tunicata and Vertebrata (or Craniata) contain luminous species but the Leptocardia (or Cephalocordata) (Amphioxus) do not. The class Pisces alone contains a very considerable number of luminous forms. The author believes all reports of luminescence among amphibia, reptiles, birds,¹ and mammals to be false or spurious, due to reflection of light or infection by luminous bacteria or to eating of such luminous insects as fire-flies as described in the section on spiders. Nevertheless, the belief that warm blooded animals, especially birds and man, are luminous has been persistent.

Among the fish both elasmobranchs and teleosts contain luminous species. Many are from the deep sea and present fascinating zoological problems. The assortment of luminous organs is as varied as among squid, and it is unfortunate that their habits in the depths cannot be readily observed. Relationship among the living vertebrates can be seen from the following classification:

Vertebrata = Craniata

Cyclostomata

Myxinoïdes (Hag-fish)

Petromyzontes (Lampreys)

Pisces

Elasmobranchii or *Euselachii* (Sharks and rays)

?*Holocephali* (?*Chimerae*)

Dipnoi (Lung fish)

Teleostomi (Bony fish)

Amphibia (Frogs, toads, salamanders)

Reptilia (Snakes, lizards, turtles)

¹ See Parker (1939) on luminous organs in lizards.

² See McAtee (1947) on luminosity in birds.

Aves (Birds)

Mammalia (Mammals)

It is often difficult to evaluate the older reports of luminescence of fish. When dead, any marine form, whether cyclostome or true fish, may serve as culture medium for saprophytic luminous bacteria. When alive and swimming, fish may stimulate the luminescence of marine dinoflagellates and appear in a fiery glow, giving rise to innumerable reports of "luminous fish" whose light really came from organisms in the sea itself. Saville Kent (1873) has called attention to this source of error, which may be the explanation of the statement by Coulon (1938) that *Orthagariscus mola*, the sun fish, is luminous.

Ryder (1880), while studying the development of fish, recorded that young porgies (*Parephippus faber*) from Mobjack Bay, Virginia, "when three days old, were very decidedly phosphorescent at night when sudden impulses were imparted to the sea water in which they were swimming about." This fish is not a luminous species, and the effect described by Ryder may have been due to luminous dinoflagellates in the water.

Probably the most famous case of a fish wrongly alleged to be self-luminous is the Bombay duck, *Harpodon nehereus*, 10 to 16 in. long, a fish of the *Scopelidae*, many of which are deep sea forms with true photophores. When salted and dried it is a delicacy eaten with curries and exported in large quantities from the west coast of India. Gunther (1880, p. 584) stated that "when newly taken its body is brilliantly phosphorescent." The fish is not a deep sea form and often frequents estuaries and rivers of Bengal and Burma. That the reported luminescence is due to saprophytic luminous bacteria growing on the dead flesh has been definitely determined by the special investigations of Kemp (1917), Prashad (1923), Hara (1934), and Haneda (1950), and this fish must be dropped from the list of luminous teleosts. Three other species of *Harpodon* are known, *H. macrochir*, *H. squamosus*, and *H. mortenseni*, the two last from depths around 1,000 ft. but they also have no luminous organs.

Another source of error in designating fish as luminous comes from eye reflection of light. Dubois (1924) treated the subject in a special paper on pseudoluminescence. The eyes of certain fish, of which the turnard (*Trigla lucerna*) is one, have a tapetum that reflects light like the eyes of a cat or a seal, giving a luminous appearance. Meinken (1934) has described fresh water fish from South Africa, *Aplocheilichthys macropthalmus* and *A. flavipinnis*, 3 to 4 in. long with enormous eyes that appear like bright dots in a semidark room.

Finally there have appeared in the literature accounts of unusual

luminous phenomenon connected with fish like that described by Möbius (1878), who caught a luminous flying fish (*Exocetus brachysoma*) near the island of Sokotura. The bright blue light shone through the skin and came from the alimentary tract which was full of small crustacea, probably ostracods, which the fish had eaten. A similar explanation of the light probably applies to another luminous flying fish caught by Weitlaner (1902). He thought the light might come from eggs of the fish, but the ingestion of luminous crustacea is a much more probable explanation.

Accounts of fish luminescence are usually to be found in monographs on fish and most general works on bioluminescence. Brauer's (1906, 08) monograph and Mangold's (1910) and Rauther's (1927) summaries are particularly good. Popular articles have been published by Zugmayer (1910), Bode (1918), Jordan (1926), and Beel (1932).

CHIMAERAE OR HOLOCEPHALI

An interesting but dubious luminescence has been reported among the Chimaeridae, of the Holocephali, that of the "Chimère arctique," the *Chimaera monstrosa* of Linnaeus, described by Risso (1810, p. 53) in his "Ichthyologie de Nice." He wrote: "The flesh of the chimera is white, sticky, with a disagreeable flavor. The soft viscous substance with which the snout is filled and which oozes from numerous pores emits a quantity of luminous rays during the night." It is not certain whether Risso referred to the dead or living animal. One might immediately designate this luminescence as bacterial infection of dead fish were it not for the existence of a number of luminous teleosts with glands that secrete luminous material and other which harbor symbiotic luminous bacteria. As no recent accounts of luminous Holocephali have appeared, the *Chimère arctique* of Risso was probably dead and probably infected with luminous bacteria.

ELASMOBRANCHII

Sharks

Observations of Luminescence. Some sharks and dogfish are self-luminous and have been studied in considerable detail. The earliest recorded observation appears to be that of Bennett (1840), who watched a living luminous shark (*Isistius brasiliensis*) alive in an aquarium for three hours. It was caught during a whaling voyage to the South Seas and called *Squalus fulgens* n. sp. Bennett described the animal as follows: "When the larger specimen, taken at night, was removed to

a dark apartment, it afforded a very extraordinary spectacle. The entire inferior surface of the body and head emitted a vivid and greenish phosphorescent gleam, imparting to the creature, by its own light, a truly ghastly and terrific appearance. The luminous effect was constant, and not perceptively increased by agitation or friction. I thought, at one time, that it shone brighter when the fish struggled, but I was not satisfied that such was the fact. When the shark expired (which was not until it had been out of the water more than three hours), the luminous appearance faded entirely from the abdomen, and more gradually from other parts; lingering the longest around the jaws and on the fins.

"The only part of the under surface of the animal which was free from luminosity was the black collar around the throat." The under surface of fins was luminous but not the upper. Bennett thought at first that "the fish had accidentally contracted some phosphorescent matter from the sea" but later decided "that the luminous power of this shark resides in a peculiar secretion of the skin" and "uniformity with which the luminous gleam occupied certain portions of the body and fins, its permanence during life and decline and cessation upon the approach and occurrence of death, did not leave a doubt in my mind but that it was a vital principle, essential to the economy of the animal."

There is no doubt of true luminescence among the sharks, for other living luminous specimens have been frequently observed, mostly at Naples or in Japan. Johann (1899) studied the histology of the skin of *Spinax niger* and a notice of luminescence by Beer was appended to Johann's monograph on the "Eigentümlicher epitheliale Gebilde (Leuchtorgane)" of this animal. Beer saw in the Naples aquarium the whole underside of an injured but living *Spinax* glow with a greenish light, like phosphorus, visible at 3 to 4 meters. It differed from phosphorus in that the light spontaneously appeared and disappeared at short intervals "but invariably increased in intensity before going out." Mechanical stimulation did not affect the luminous and non luminous regions, but electrical stimulation strong enough to cause contraction of muscles caused the light to appear. When moribund, with no light visible, electrical stimulation of the spinal cord did not again excite the luminescence of Beer's specimen.

Burckhardt (1900) also had seen the light of *Spinax niger*, again collected at Naples. By examining the skin of other selachians for luminous organs, Burckhardt established the ability to light from preserved material of quite a number of sharks. These included *Laemargus* (*Somniosus*) *rostratus*, *L. borealis*, *L. brevipennis*, *Spinax pusillus*, *S. granulosus*, *Isistius brasiliensis*, *Euprotomiscus Labordii*, *Centro-*

scyllium granulatum, *C. Fabricii*, and *Paracentroscyllium* (*Centroscyllium*) *ornatum*. No photogenic organs were found in *Centrophorus granulatus*, *C. calceus*, *C. squamosus*, *Scymnodon ringens*, *Sevini*, *lichia*, *Centrina*, *Notidanus*, *Echinorhinus*, or *Chlamydosclache*. Each genus and species has its particular distribution of luminous organs although the pattern in two related species may be quite similar.

In Japan Ohshima (1911) observed the light of *Etmopterus lucifer* and *E. frontimaculatus*, closely allied but with a different photophore distribution, and Schmidt (1930) observed *E. pusillus*. Ohshima's description of *E. frontimaculatus* is as follows:

"In ventral aspect, nearly the whole surface of the fish could be seen as a faint, whitish, phosphorescent body, when a proper stimulus was applied to it. At the bases of paired fins, in the postanal region and in two discontinuous parts of the caudal area, the luminosity was somewhat stronger, while the eyes, the mouth, the mandibular spaces . . . the anal region, and the peduncular part remained dark. On the dorsal side of the fish I have not been able to observe any luminescence.

"The light was quite tranquil and not flaring, and it must be stated in particular that spontaneous luminescence has never been observed.

"When the fish was held in one hand and was pressed or rubbed with the other, the luminescence was not immediately called forth but became apparent after some minutes, the light gradually appearing or vanishing or attaining maximum intensity here and there at different places.

"Stimulation with ammonia water did not show any effect on luminescence. . . .

"The facts that no sudden change of luminosity takes place and that there is such a local difference in the intensity of the emitted light may, in some cases, be due to the action of the pigment cells which form what I have called the 'iris.' When contracted, they allow the exit of the light produced in the photogenic body lying underneath, while their expansion makes the iris act as a screen that shut in the light.

"If it be, as Johann has made out in *Spinax niger*, that the organ is not specially innervated, this may be in relation with the fact that mechanical stimulation does not cause immediate change of the luminescent phenomena."

Finally Hickling (1928) has frequently observed the luminescence of the dogfish, *Spinax niger*, taken from the trawl catch. "When newly caught and vigorously alive, *Spinax* often shows no luminescence, nor are dead specimens luminescent. But while moribund, *Spinax* may show a greenish blue light very distinctly visible to the dark-adapted eye.

"Spots and streaks of light appear on the back of the fish, but their effect is very feeble compared with that of the belly, which appears as a steadily-glowing sheet of light, with brighter areas about the mouth, on the pectoral fin-bases, in the pelvic region, and on the tail fin. . . .

"When a luminescent specimen is held so that one's line of vision is perpendicular to the ventral surface of the fish, the luminescence is plainly visible. When the fish is then rotated slightly to left or right about its long axis, the light disappears. This observation seems to offer some explanation of the function of the luminescence of *Spinax*.

"The complex lantern-like structure of each individual organ seems designed to throw out a parallel beam of light, and to prevent scattering of the rays: the arrangement of the axes of all the organs parallel to the median vertical axis of the fish, seems to aim at precisely the effect described above, namely, that the luminescence will only shine upon objects immediately beneath the ventral surface.

"The mouth of *Spinax* is situated remarkably far behind the tip of the snout, so that *Spinax* can obviously only seize objects immediately beneath (in the relative sense) its mouth. But it is only when an object is immediately below the ventral surface of the fish that the light from the luminous organs flashes fully upon it. One may therefore suggest that the sudden flash of light, at the moment of attack, may cause the prey of *Spinax* to hesitate for just that fraction of a second in which the mouth can make a successful snatch." The position of these genera in a classification of elasmobranchs by C. T. Regan and J. R. Norman is as follows:

Elasmobranchii

Pleurotremata (sharks, dog-fish)

Notidanoidea

Chlamydoselachidae

Hexanchidae

Galeoidea

Odontaspidae

Lamnidae

Orectolobidae

Scylliorhinidae

Carcharinidae

Squaloidea

Heterodontidae or Cestraciontidae

Pristiopharidae

Squalidae (including *Laemargus* or *Somniosus*, *Isistius*, *Euprotomicrus*, *Centrosyllium*, *Paracentrosyllium*, *Etmopterus* or *Spinax*)

Squatinae

Hypotremata (skates, rays, torpedos)

Narcobatoidea

Torpedinidae (including *Benthobatis*)

Batoidea

Rhinobatidae

Pristidae

Raiidae

Trygonidae or Dasybatidae

Myliobatidae

Molbulidae

Histology. Structure of the light organs of selachians has been investigated by Johann (1899), Burekhardt (1900), Dahlgren and Kemper (1908), and Ohshima (1911). At the time Johann's study was made, he was unaware that the luminescence of the "peculiar epidermal structures" of *Spinax* had been seen and he designated them as luminous organs on purely structural grounds. Leydig (1903) who had previously (1879, 81) described eye-like organs, structures like glass beads and luminous organs in the skin of teleost fishes, hailed the paper of Johann as undoubtedly indicating their function to be luminous, but questioned the exact significance of luminous organs, whether they should be classified as glands or as sense organs.

Actually the tiny photophores of sharks do not secrete a luminous fluid to the outside. No luminous secretion adheres when handling the animals. In the Japanese shark, *Etmopterus*, the punctuate or circular photophores are only 100 μ in diameter, and the linear photophores are about 300 μ in length by 100 μ broad. The latter are to be regarded as fusions of the punctate photophores. These are hemispherical cup-shaped epidermal swellings made up entirely of epidermal cells, some of which have assumed the function of light production while others have been modified to form crude lenses. Pigment cells surround the photophore, preventing light from striking the internal structures of the fish and a ring of pigment cells exterior to the hemispherical group of photogenic cells forms a sort of "iris," which by contraction and expansion presumably control the light. A longitudinal section of the photophore of *Spinax* (or *Etmopterus*) is reproduced in Fig. 165. These primitive light organs of selachians illustrate the first stages in evolution of the complicated and highly efficient photophores of teleosts. The fact that selachian luminescence is found mostly on the ventral side of the fish has led to the idea that the light is used to illuminate the sea bottom. However, the luminescence is very weak and the pattern of organs, different in each species, suggests the equally probable or more probable function of a recognition mark. The view of Hickling on use of the light has already been mentioned.

Physiology and Biochemistry. Practically all that is known of the

physiology of light production in selachians has already been presented. The light is not continuous and seems to be decidedly independent of stimulation. No bacteriological or biochemical studies have as yet been made but should be started at the first opportunity. It is also important to determine microscopically if all changes in light intensity are con-

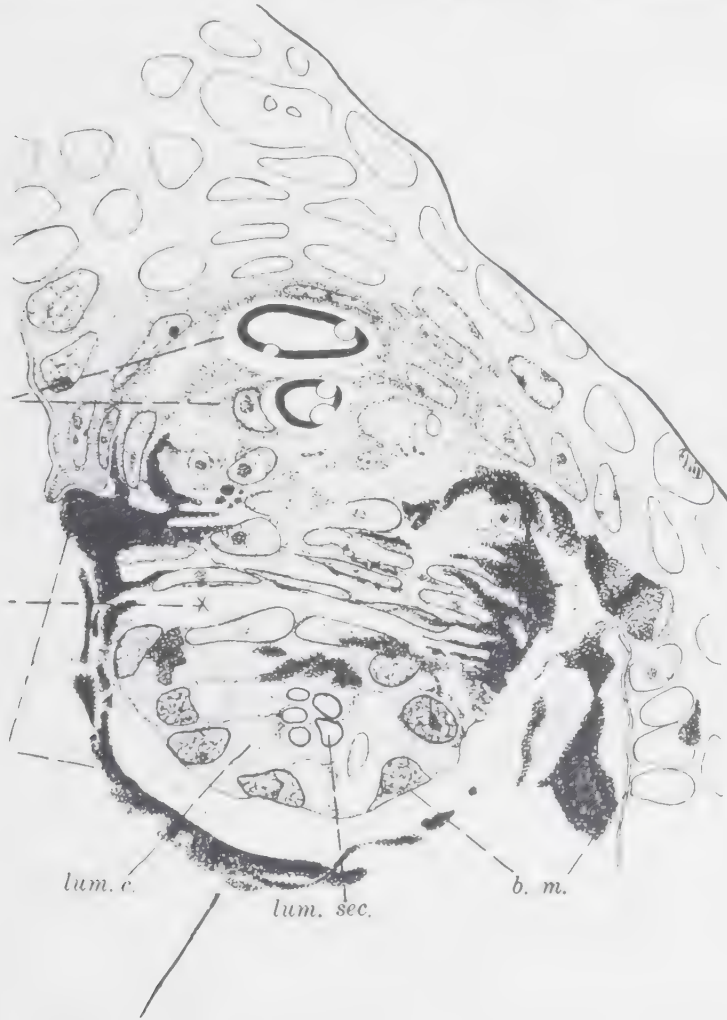


FIG. 165. Section of the luminous organ of a shark, *Spinax niger*, showing black pigment cells; lum. c., luminous cells; lum. sec., luminous secretion; b. m., basement membrane. After Dahlgren and Kepner.

rolled by pigment cell movements as Ohshima (1911) has suggested. Another explanation of the slow onset of luminescence is possible and should be tested. The light may be hormone-controlled, as in the California toadfish (*Porichthys notatus*), and the deep sea fish *Echnostoma ctenobarba*, excited by adrenaline injection. Adrenaline may also function in selachians and if so, the question arises as

to whether it acts directly on light organs or indirectly on the chromatophores.

Rays

The only ray which has been described as luminous was caught by Alcock (1902), an electric ray from 430 fathoms depth off the Travancore coast of India. The eyes of the animal, *Benthobatis moresbyi*, were small and rudimentary and Alcock stated "Curiously enough, this ray, though it must be quite blind, has a row of minute luminous glands along the edge of its disk, these probably being lures to attract prey."

TELEOSTOMI

Accessory Eyes

It is rather surprising to find that the older literature contains few observations on the luminescence of living bony fish. Even though most luminous species are deep sea forms, they do occasionally come to the surface, and their photophores are prominent skin structures, known to Risso (1820) and Valenciennes without a realization of their function. Cocco (1838) was probably the first to speak of them as "lucidi" or "punti luminosi," but the observation may have referred to reflected light. Orioli (1850) wrote of "pesci e del mare che rilucono nella oscurita." K lliker (1853) thought the skin spots were sense organs and the great Leuckart (1865) designated the photophores of *Chauliodus sloani* as accessory eyes (Nebenaugen), although he could not demonstrate a nerve or anything like a retina. They are shown in Fig. 166. Lereboullet (1864) suggested that a complete histological investigation should be made and both Ussow (1879) and Leydig (1879, 81) carried this out. Ussow studied a number of forms at Messina in 1872 and held that the skin spots should be placed in two categories. In *Chauliodus*, *Stomias*, and *Astronesthes* they were modified eyes like those of *Euphausia* and the worm, *Polyopthalmus*, while in *Gonostoma*, *Maurolicus*, and *Scopelus* they were modified slime glands. *Sternoptyx* and *Argyropelecus* were intermediate forms with both types. Ussow published figures of sections of the organs and a table showing the distribution in different fish.

A great deal of attention was paid to these pearl like spots of fishes in the 1880's. In his first paper, Leydig (1879) became skeptical of their function as accessory eyes and mentioned a letter which Willemoes Suhm (1875) wrote from the *Challenger* to Prof. von Siebold in which he spoke of a *Sternoptyx*, which was "like a luminous star as it hung in the net at night." Later, Leydig (1881) expressed his views in a book. He studied *Gonostoma*, *Ichthyococcus*, *Argyropelecus*, and

six species of *Scopelus* and divided the organs into three types: I. Eye-like (Augenähnliche) organs; II. Glass bead-like (Glasperlenähnliche) organs, and III. Light organs (Leuchtorgane). The first type looked like eyes but were very different. The second and third types were still more different from eyes, and Leydig thought there was more ground for believing them to be electric organs than sense organs. He even considered the possibility of a sixth sense. Nevertheless, on the whole but with some hesitation, Leydig designated them light organs of a special kind but not glandular. Their mysterious nature aroused

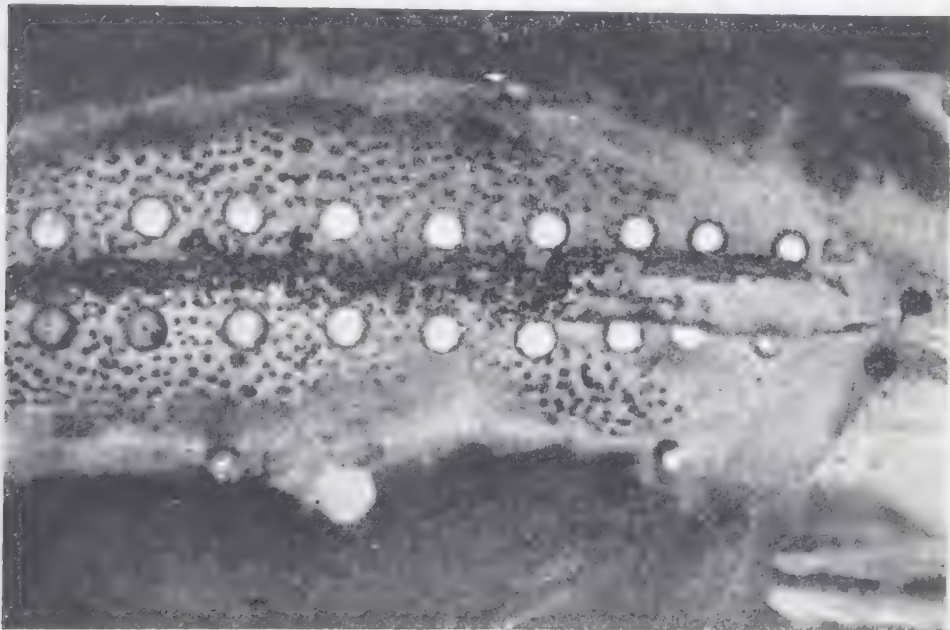


FIG. 166. The double row of "pearly spots" or photophores of *Cyclothone*. Photo by Wm. Beebe.

considerable popular interest and articles on the subject appeared in *Kosmos* by Kraus (1881) and in the *Popular Science Review* by Bell (1881). The Leydig book stimulated the search for other fish with "Nebenaugen," and Solger (1881) described a *Porichthys porossissimus* preserved in the Godeffroy Museum with many such structures which he labeled light organs, and figured a rather crude section of one of them.

Observations of Luminescence

Despite the controversy over photophores of deep sea fish an increasing number of observations on light production was accumulating. The various references have been collected by Brauer (1908, p. 127-33). In addition to the observation of Willemoes-Suhm (1875) and others on the *Challenger* previously referred to, Reinhardt (1854) had

described an *Astronesthes fieldii* obtained during a voyage to Brazil, which "sent forth two strong and vivid greenish lights, which intermitted momentarily and ceased altogether when the fish died." A second specimen flashed from "a spot on the forehead a little before the eyes." Guppy (1882) noted a faint light in the pearly organs of *Scopelus* which was gasping and about to die. Only those pearly organs in the pectoral region were luminous. Prince (1892) held that the two rows of discs on the sides of deep water *Maurolicus*, sometimes driven ashore in England by storms, must be phosphorescent organs as the fish had two eyes with which to see. Grassi (according to Chiarini and Gatti, 1899), members of the "Valdivia" expedition in 1898-99, Mangold (1907), Kiernik (1908), Ohshima (1911), Beebe (1926, 34, 44), Skowron (1928), and many others, including the author (1931) have seen the light. There is no doubt of the photogenic function of the pearl-like organs of deep sea fish and of similar photophores on the skin of some surface fish, such as those of the California toad-fish, *Porichthys notatus*, whose luminescence has also been observed by many.

Distribution of Luminous Deep Sea Fish

The great hauls of deep sea fish made by many oceanic expeditions have revealed an extraordinary variety of light organs, quite comparable to and even surpassing the types among the cephalopods. As contrasted with surface fish, the deep sea forms, with some exceptions, have many definite photophores, the pearly organs of Ussow and Leidig, arranged in a pattern, largely on ventral or lateral surfaces of the fish. The *Lightning* and the *Porcupine* in 1868 and 1870, pioneering survey ships in deep sea investigation, obtained mostly bottom invertebrates and proved the existence of life at great depths. Bathypelagic ichthyology begins with the *Challenger* expedition in 1873-76. The *Challenger* fishes were identified by Gunther (1880, 87), who listed 370 fishes known to live below the 100 fathom line and 23 fish at depths between 2,000 to 2,900 fathoms. Before the *Challenger* expedition only 30 "deep sea" forms were known. A large proportion of the *Challenger* fish possessed photophores, whose structure was studied by von Ledenfeld (1887). He investigated eleven species of the genera, *Opisthias*, *Echiostoma*, *Pachystomias*, *Malacosteus*, *Astronesthes*, *Argyropelecus*, *Sternoptyx*, *Scopelus*, *Xenodermichthys*, and *Halosaurus*.

Valliant (1888) dealt with deep sea fish obtained by the *Travillier* and *Talisman* in 1880-83, but not much attention was paid to luminous organs. The same is true of Goode and Bean's (1895) "Oceanic Ichthyology," based on early collections of "Blake," "Albatross" and "Fish Hawk." At this time some 600 deep sea fish (living below 1,000

ft.) were known and a number of luminous forms were systematically described, but there is no special section on luminescence. Garman (1899) and Gilbert (1903, 08, 15) have also studied the luminous fishes of later "Albatross" collections and added many new species to the literature.

The Indian survey ship, *Investigator*, obtained during the nineties many luminous deep sea fish in the Indian Ocean, described by Alcock (1892-1908), and the *Siboga* expedition to Netherlands India in 1899-1900 added to knowledge of luminous fishes, which were described by Weber (1902, 13).

It was the *Valdivia* expedition of 1898-99 which obtained material for the great monograph of Brauer on Tiefseefische, adding greatly to knowledge of photophores. Later expeditions also collected a very large number of deep sea fish for systematic work. These collections cannot be considered in detail, but in addition to studies already mentioned much useful information will be found in the publications on the Prince of Monaco's collections (Collet, 1896; Zugmayer, 1911; Roule, 1919; Nussbaum-Hilarowicz, 1920, 23; Roule and Ansel, 1933), the British Museum collection (Regan, 1923), *Discovery* Reports (Norman, 1930), the Museum of Comparative Zoology (Borodin, 1931), the U.S. National Museum (Schultz, 1938; Parr, 1927-37), *Dana* expeditions (Regan and Trewavas, 1929, 30; Tåning, 1932), the deep sea fish of South Africa (Barnard, 1925) and Japan (Matsubara, 1938), and many others, particularly the studies of Beebe and Crane at Bermuda, described below.

Brauer's monograph is divided into a systematic (1906) and an anatomical (1908) part, the latter forming a comprehensive treatise on fish luminescence. Among both surface and deep sea teleosts, Brauer mentioned 228 species in 63 genera known to be luminous. If the 400-meter line is accepted as the level where the depths begin, 1,007 species in 309 genera are deep sea forms. Among them a maximum of 112 species (11%) in 37 genera and a minimum of 62 species (6%) in 20 genera are luminous.

This low estimate has been criticized by Weill (1938) as giving a somewhat erroneous impression. First, Brauer set his depth line at 400 meters, which would eliminate many deep sea fish which are caught at the surface when swirled up from the depths or when they come to the surface at night. Second, Brauer was concerned with species and genera, whereas Weill held that number of individuals was more important. The nets of the *Valdivia* caught 3,169 specimens of fish, representing 184 species of 93 genera) among which 2,805 individuals (representing 91 species of 36 genera) had luminous organs.

i.e., 88% of the individuals. Based on Brauer's minimum of 62 species (in 20 genera) of truly deep sea luminous forms, the number of individuals caught was 2,194 in a total of 3,169 or 78% of the whole. Thus the prevalence of luminous individuals among deep sea fish is high.

A later estimate has been given by Beebe (1937), based on a preliminary list of deep sea fish (excluding surface and bottom forms) caught over a period of nine years within an 8-mile circle south of Bermuda. Over 1,500 hauls were made with a 1-meter diameter net at depths from 400 to 1,400 fathoms. Although only half of the captured fish had been monographed in 1937, Beebe wrote, "We have taken more than one-third of all the corresponding abyssal fish known to science. More specifically from this extremely limited area we have brought up fishes representing at least 10 orders, 46 families, 65 genera, 220 species and 115,747 individuals, almost all from depths below 300 or 400 fathoms in strictly abyssal habitats. Thirty one of these species have been described as new." Beebe found that members of the genus *Cyclothone* of the Gonostomidae were most numerous. "If we consider only two species, *C. microdon* and *C. braueri*, . . . we find that in total numbers they compose four-fifths or 82% (94,684) of all the other deep sea individual fish together. . . . Considering number of individuals the following families are in the lead: Gonostomidae, Myctophidae, Sternoptychidae, Melamphaeidae, Chauliodontidae, Paralepididae, Maurolicidae, Melanostomiidae, Aceratiidae, and Serrivomeridae. With the species of these 10 families amounting to only 60% of all, we find that the individual count comes to 98.8% of all the fish taken.

"Luminosity is present as follows: in 50% of the orders, 39% of the families, 81% of the genera, 66% of the species, and (thanks again to *Cyclothone* and *Myctophidae*) to 96.5% of all individuals."

This Bermuda study of Beebe and his collaborators is the greatest concerted effort to sample the deep sea fauna of a restricted region and should give a highly accurate picture of the abundance of luminous species. Systematic descriptions of the fishes themselves will be found in the publications of Beebe (1932, 35) and Beebe and Crane (1936, 39), in a series of papers appearing in *Zoologica*.

Beebe (1924) also observed luminous fish in his bathysphere descents and actually described three new species. The author (1939, 48) has attempted to photograph these deep sea forms with an automatic moving picture camera and automatic lighting devices in a pressure chamber, suspended freely at depths from 500 to 1,320 fathoms in the same region south of Bermuda. Despite the taking of 17,000 individual frames, not a single fish appeared in any picture.

The method is somewhat like pointing a camera at the sky hoping to obtain a picture of a bird, but it is rather surprising that no fish was recorded. The element of complete chance was eliminated by hanging a lure in the water before the camera, a model of a deep sea fish with luminous spots painted on its sides with luminous paint. Perhaps the deep sea fish easily recognized the deception, or perhaps the light, which suddenly flashed on and off, or the noise of motors and clicking of relays scared the fish away. Photographs made with the Ewing camera³ indicate an abundant invertebrate life on the sea bottom at great depths.

An early recognized peculiarity of distribution among deep sea luminous fish is absence of bottom forms. Brauer has stated that no luminous deep sea bottom fish are known—they live in the twilight zone or just below. This statement may be extreme but even angler fish are not benthonic in habitat. They live at medium depths from 500 to 5,000 meters. At great depths the eyes of fish, like that of euphausiids, tend to become rudimentary and light organs are reduced in size.

An interesting and well-known fact is the wide geographical distribution of bathypelagic species. Living as they do in a stratum of sea without light and with a constant low temperature around 4°C, they are not subjected to climatic barriers, and a species caught in Bermuda may also be found in the North and South Pacific, the North and South Atlantic, the Indian Ocean, or the Arafura Sea.

Classification

Of the many proposed classifications of teleosts the author has selected one which does not subdivide too extensively and at the same time brings out the various relationships. The genera and families of living fish are so numerous that in most cases only orders can be tabulated, with the families and genera containing luminous species in italics. The classification of Regan and Norman follows:

Teleostomi

Palaeopterygii

Cladistia

Polypteridae

Calamoichthyidae

Chondrastei

Acipenseridae

Polyodontidae

³M. Ewing, A. Vine, and J. L. Worzel, "Photography of the Ocean Bottom," *J. Opt. Soc. Amer.*, 36, 307-21, 1946.

Neopterygii

Protospondyli (Amiidae)

Ginglymodi (Lepidosteidae)

Isospondyli

Clupeioidea (8 families, including *Alepocephalidae* with *Xenodermichthys*, *Photostylus*, *Bathytroches*, *Searsia*, Binghamia, and probably other luminous genera)

Salmonioidea (9 families, including *Argentinidae* with *Bathylagus*, and possibly other luminous genera)

Stomiatoidea (see classification under Isospondyli and Iniomi)

Osteoglossoidea (2 families)

Notopteroidea (2 families)

Mormyroidea (2 families)

Gonorrhynchoidea (Gonorrhynchidae)

Haplomi (3 families)

Iniomi

Mycetophioidea (see classification under Isospondyli and Iniomi)

Alepidosauroida (Scopelarchidae, Cetomimidae, Alepidosauridae, Omosudidae)

Ateleopoidea (Ateleopidae)

Giganturoidea (Giganturidae)

Lyomeri (Saccopharyngidae and Eurypharyngidae)

Ostariophysi

Cyprinoidea (14 families)

Siluroidea (22 families)

Apodes (22 families)

?*Heteromi* (3 families, including ?*Halosauridae* with ?*Halosaurus*)

Synentognathi

Scombresocoidea (2 families)

Exocoetoidea (2 families)

Microcyprini (3 families)

Salmopercae (2 families)

Solenichthyes (6 families)

Anacanthini (*Macruridae*, Merluccidae, *Gadidae*, Muraenolepidae)

Allotriognathi (5 families)

Berycomorphi (11 families, including *Monocentridae* *Anomalopidae* and ?*Melamphaeidae*, with ?*Melamphaes*)

Zeomorphi (2 families)

Percomorphi (124 families, including *Acropomatidae* *Serranidae*, *Leiognathidae*, ?*Gemphylidae* with ?*Ruvettus*, ?*Zoarchidae* with ?*Bassozetus*, ?*Brotulidae* with ?*Leucicoris*, ?*Lamprogrammus*, ?*Mironus*)

Scleroparei (21 families)

Hypostomides (Pegasidae)

Heterosomata (5 families)

Discocephali (Echeneididae)

Plectognathi (8 families)

Melacichthyes (Icosteidae)

Xenopterygii (Gobiesocidae)

Haplodoci (*Batrachoididae*)

Pediculati (see classification under this order)

Opisthomi (Mastacembelidae)
Synbranchii (2 families)

Deep sea fish with many photophores belong mostly in the Isospondyli and the Iniomi. There are in addition the deep sea forms bearing tentacles or illicia with a luminous bulb, the Pediculati, and a number of luminous surface fish or fish from deeper water which come to the surface to breed. As can be seen from the classification, the other surface species are scattered in various unrelated families.

Fossil Luminous Fish

The previous classification contains only families living in modern times. The number of known fossil fish is very large, and it is not surprising to find that there are many fossil forms among the Stomiatoidea and Iniomi and that occasionally fossilized photophores can be detected. For example, Arambourg (1920) has described these as small hemispheric granules on the scales in *Myctophum prolaternatum*, n.sp. from the Sahara region near Oran. *M. prolaternatum* is closely allied to the recent *M. laternatum*, widely distributed in the Indian Ocean, western Pacific, and eastern Atlantic waters from Morocco to the Gulf of Guinea.

Among the presumably luminous stomiatoids, Pauca (1929) has described from the oligocene, *Mrazecia mrazeci*, different from all living genera and most nearly like *Gonostoma denudatum*. He also (1931) found *Sternoptyx prisca*, about 1 in. long and quite similar to the living *S. diaphana*, from the oligocene of Platra-Neamt. Romer¹ has listed *Chauliodus*, *Cyclothone*, *Gonostoma*, *Photichthys*, *Argyroleucus*, many genera of the *Myctophidae* and the pediculate, *Histionopterus*, of the *Antennariidae* as containing fossil species. The British Museum list of Regan and Norman contains the fossil stomiatoid family, *Enchodontidae*.

Teleosts of Doubtful Luminosity

A few of the alleged luminous genera are dubious, as the photogenic function has been inferred from study of structures in preserved material. *Xenodermichthys* of the *Alepocephalidae*, suborder *Clupeoidea*, described by Gunther (1887) from the *Challenger* collection has "very small raised nodules with whitish center distributed over the body. . . . I consider the organs to possess luminous properties." The structure was sectioned and studied by von Lendenfeld (1887) who also thought its function luminous. It was made up of a proximal and distal part with atypical phosphorescent cells. A pigment layer

¹ A. S. Romer, *Vertebrate Paleontology*, Chicago, 1945.

covered the proximal region and a large nerve entered the organ. Rauther (1927) has interpreted the nodules as skin sense organs of a peculiar form, but Beebe (1933) has referred to them as photophores and has also described the photophores on the lower jaw of *Bathytrochus rostratus*, on the eyeball and ventral surface of *Dolichopterygion biocularis*, and the stalked photophores of *Photostylus pycnopterus*, all belonging to the Alepocephalidae.

The group of fish which possess what von Lendenfeld called "radiating discs" are of very doubtful luminescence. These belong to the genera *Halosaurus*, *Bassozetus*, *Leucicorus*, *Macrurus*, and *Ipnotops*. In *Halosaurus macrochir* and *H. radiatus* of the Halosauridae, order Heteromii, von Lendenfeld (1887, 1905) had described the luminous structures of glandular character under the gill covers and radiating discs on the scales of the lateral line system. The latter have a high columnar epithelium over a layer of blood vessels and a large nerve supply. They lie in a pocket of the scale, which opens to emit the light. Richard (1910) also has called these structures light organs but Rauther (1927) interpreted the radiating discs of the Halosauridae as well as similar ones in *Macrurus canis* (see von Lendenfeld, 1905) as sense plates of the lateral line system.

Among the Brotulidae and Zoarchidae of the Percomorpha, *Bassozetus nasus*, *Leucicoris lusciosus*, *Lamprogrammus niger*, and *Mixonius* have dubious light organs. Von Lendenfeld (1905) described the radiating discs of *Bassozetus* and *Leucicoris* as made up of a number of deep depressions on the head. They contain a connective tissue thickening of the membrane close to the base, covered with a reticulum of pigment cells and a rich nerve supply. The columnar cells characteristic of *Halosaurus* are absent.

The radiating discs of *Macrurus canis* are situated on the floors of the cephalic slime canals, made up of white patches with pigment cell reticulation and a nerve supply, but the fine structure could not be made out. Rauther (1927) believed these structures as well as the enlarged scales of *Lamprogrammus* to be part of the lateral line sensory system.

Finally the scopolid fish, *Ipnotops murrayi* and *I. agassizi* of the Ipnotidae aroused considerable interest because of large dorsal plate-like structures on each side of the flat head. Murray and Gunther had considered those of *I. murrayi*, obtained by the *Challenger* in 1,507 fathoms, phosphorescent organs, and Moseley (1887) came to the same conclusion from a histological study. They are made up of long columnar cells with nuclei at the distal end lying above a rich plexus of blood vessels. The columnar cells are arranged in hexagonal groups

separated by pigment. Branches of the trigeminal innervate the organ. Von Lendenfeld (1905), from his study of *I. agassizi*, caught by the *Albatross* in 1,360 fathoms, also considered the structure to be a luminous disc organ, certainly not an eye and Brauer (1908, p. 126) pointed out that the structure was quite different from that of rudimentary eyes and not supplied by the optic nerve. However, the disc is also very different from the light organs of other scopoloid fish and Rauther (1927) has held that the possibility of a peculiar photosensitive organ has not been disproved.

Distribution of Light Organs

Despite the enormous diversity of luminous teleosts, a few generalizations can be made. Like the squid, fish luminescence may be bacterial or intrinsic, and it seems to be true among both squid and fish that structures serving for the growth of luminous bacteria have a canal or pores opening to the outside sea water. As luminous bacteria are only rarely extruded in any quantity and then by pressing on the organ, we may suppose the openings function either to allow bacteria to enter the gland at a certain stage of development or to permit dead bacteria to pass out or both.

Bacterial light organs are usually found in surface or medium depth fish and are relatively simple, either single or paired. Their light shines continually but may be decreased or completely excluded by secondary control, as by chromatophores, or movable screens, or by shifting the position of the organ.

Truly deep sea fish, on the other hand, present the opposite picture to the one described above—their photophores are numerous and highly complex in structure, with lenses and reflectors. The light appears only on stimulation, although the response may not be as sudden as in the case of such invertebrates as *Pyrosoma*, *Pennatulula*, or *Noctiluca*. Frequently the photophores are completely closed, but some have open ducts and some have remnants of ducts with no lumen and no outside connection.

An early and important observation has to do with the position of the photophores. It was found that these organs are constant in number and specific in position in each species of deep sea fish, hence of enormous value in classification. This is particularly well seen among the many species of the genus *Myctophum*, studied by Brauer (1904), who gave names to the various groups of photophores in different parts of the body. Frequently there will be a slight difference in position of photophores in male and female so that they could be of value as distinguishing marks.

Although symbiotic luminous bacteria as a source of light are not to be expected in the closed organs, few actual bacteriological studies have been made. The author knows of only one published attempt to test for luminous bacteria in these forms, that of Haneda (1950) from *Yarrella* of the Gonostomatidae and *Polyipnus* of the Stenopodidae, both with closed photophores. These fish are mostly caught by trawlers in deep water but occasionally come to the surface at night. According to Haneda the organs luminesce on stimulation, and the control is not by chromatophores or screens of any kind. Despite extensive bacteriological examinations no bacteria could be demonstrated.

As in the case of squid it is more convenient in discussing teleost luminescence to discard evolutionary relationships and to divide the light organs of fishes into the two previously discussed categories. I. Single or paired open organs with luminous bacteria; II. Skin photophores with or without a duct. The open and closed variety may be present in the same fish. At present the fish in group II are not known to contain luminous bacteria, but as further investigations are made, some species may be shifted to the first group.

Fish with Open Glands Mostly Containing Luminous Bacteria

A considerable number of unrelated fish belong in group I. Their open glands contain luminous bacteria, many of which have been isolated in pure culture. In some cases the bacteria form a luminous slime on the belly when the fish is gently pressed. In other cases, there is no indication that they come out of the gland, despite the presence of a duct or pores. In a few species, the luminous organ is provided with a very definite reflector which scatters the light over a large area of the body, resulting in what Haneda (1950) has called an "indirect emission luminous organ." Many of these luminous fish have been known to local fishermen long before they were studied scientifically.

In addition to the fish which have been thoroughly investigated and in which luminous bacteria have been isolated, the deep sea angler fish of the *Pediculati*, possess lures, open glandular luminous organs at the tip of their tentacles which have every appearance of containing luminous bacteria, although proof of this is at present lacking. Nevertheless they will be considered in group I and the *Saccopharyngidae* will also be considered in group I, although little is known of the luminous organ.

Finally there is another possible fish to be included in the group, the eel fish, *Ruvettus pretiosus* of the *Gempylidae*, first described by

Cocco (1829) from Messina. Nothing is known of the luminous organ, but Gudger and Mowbray (1927) described one caught by a fisherman at Bermuda in 75 fathoms, which "when it came to the surface, appeared to be surrounded by a large ball of blue fire." This specimen was 4 ft long and weighed 24 lb. The oil-fish is rather rare, but there is another account of its luminescence in Felipe Poey's history of Cuba, written in Spanish in 1854. Speaking of the oil-fish, Poey wrote: "When one sees it on the surface of the water it is surrounded by a luminous or phosphorescent globe."

Anacanthini, Macrouridae. The first indication that macrourids were luminous came from a note of Osorio (1912), who described an unusual method of catching fish by the fishermen of Cezimbra, Portugal. As bait they take a fragment of dog-fish flesh and rub it on the belly of *Malacocephalus laevis*, from which there issues from an opening near the anus a "thick yellow turbid liquid which phosphoresces with a light like that of the blue sky." The light of this bait, which lasts many hours, attracts fish to their fish hooks. Osorio found that the light from the liquid would affect a photographic plate and declared that luminous bacteria were present, but he made no cultures.

The liquid comes from a large gland, which Gilbert and Hubbs (1912, 20) had found in many genera and used in classification of macrourid fishes. They suggested that it might be a phosphorescent organ. Detailed luminescent studies of *Malacocephalus laevis* have been made by Hickling (1925, 26), Haneda (1938), and Imai (1942), and of *Coelorrhynchus coelorrhynchus* by Hickling (1931). Yasaki and Haneda (1935) and Haneda⁵ have found the luminous gland present in the following species:

<i>Eoryphaenoides garmani</i>	<i>C. tokiensis</i>
<i>Eoryphaenoides misakius</i>	<i>C. hubbsi</i>
<i>Abyssicola macrochir</i>	<i>Nezumia condylura</i>
<i>Coelorrhynchus anatirosuris</i>	<i>Malacocephalus laevis</i>
<i>C. japonicus</i>	<i>Hymenocephalus striatissimus</i>
<i>C. kishinouyei</i>	<i>H. kuronumai</i>
<i>C. parallelus</i>	<i>H. gracilis</i>

Hickling (1931) reported no trace of a gland in *Trachyrhynchus trachyrhynchus*, but the probable luminous organ of *Hymenocephalus italicus* had been described by Trotti (1936).

In his first paper on *Malacocephalus laevis*, caught on the outer edge of the continental shelf of western Europe from Ireland to Morocco, Hickling described the gland as lying in front of the rectum, between and behind the pelvic fins, oval in shape, and bound in

⁵ Unpublished.

connective tissue. It tapers posteriorly to a duct that opens just anterior to the rectum, as shown in Fig. 167. There are structures present which might be regarded as reflectors, but Hickling doubted that function because the gland is surrounded by black pigment, has a duct, and definitely secretes a viscid bluish luminescent secretion which is very apparent on the belly of fish removed from the trawl at night.

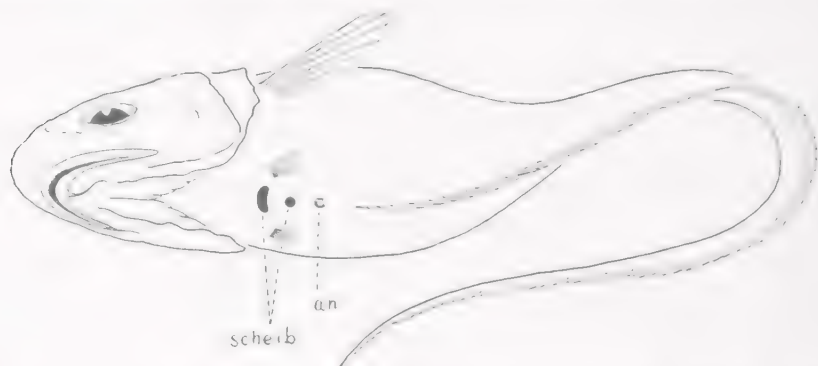


FIG. 167. *Malacocephalus laevis*, showing anus (an), disk (scheib), and opening (in middle) of luminous gland. After Haneda.

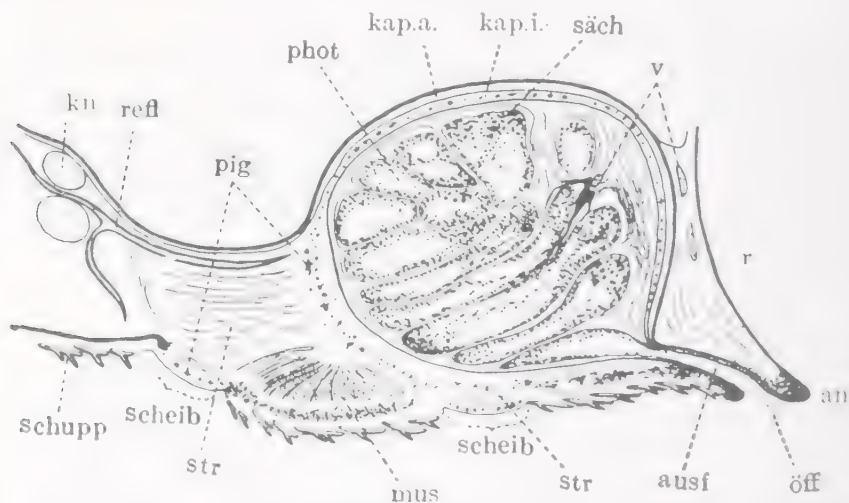


FIG. 168. Section of the luminous organ of *Malacocephalus laevis*, showing photogenic tubules (phot), duct (ausf), and opening (öff). After Haneda.

The gland is richly supplied by blood vessels, but there appears to be no special nerve supply, certainly not from the rectum. However, there is "a network of nerve cells in the connective tissue capsule of the gland, from which fibers pass into the folds of tissue which project into the lumen, as shown in Fig. 168. This network seems to be supplied by nerves arising from the dermis below the gland."

According to Haneda (1938) a faint light can be seen coming from

the gland when the ventral surface, particularly the elongated disc is observed. The luminescence varies, depending on conditions of water temperature, light, etc., because of expansion or contraction of chromatophores on the ventral side of the organ.

When squeezed in daylight, the fish "yield a large viscid drop of greenish yellow slime," full of cell fragments and particles. In 1925, Hickling, from sections of the gland and staining reactions interpreted these as granules, not bacteria. In sea water the granules form a suspension with "bright greenish blue phosphorescence" which soon becomes dark except at the top in contact with oxygen. Shaking is necessary to keep the light uniform in the tube so that a considerable oxygen consumption is indicated. The light was very persistent. Three drops of secretion in a canvas bucket of sea water remained luminous for twenty-four hours.

The light of a drop of secretion in distilled water went out immediately, but "if the secretion be first dissolved in a small quantity of sea water and then diluted with distilled water to almost any extent, there is neither extinguishing nor brightening of the light, the new solution having the same appearance as a corresponding solution in sea water. The glow soon fades out, however; the exact time is uncertain, but it is usually between eight and twelve hours."

Hickling also found that the secretion could be mixed with sugar or $MgSO_4$ crystals and would still luminesce brightly when sea water was added, another fact which spoke against the bacterial nature. A luciferin-luciferase reaction was also obtained. As this reaction cannot be demonstrated in luminous bacteria, the evidence seemed to indicate self-luminescence in this fish. There is, however, one possible source of error in carrying out the luciferin-luciferase test. If the "luciferin solution prepared by heating the secretion until the light disappeared" had not been thoroughly cooled and shaken before adding it to the "luciferase solution," Hickling may have observed merely the return of light after heated luminous bacteria are cooled and aerated.

In a second paper Hickling (1926) described examination of the fresh secretion with a microscope. It is made up of "globes," 30 to 40 μ in diameter enclosed in a cytoplasmic sheath, together with fragments of various types of cells. The sheath breaks, liberating the highly refractive oval granules, between 1 and 2 μ in length and with a "distinct greenish fluorescence. They seem to have a fluid interior bounded by a membrane. In my view these granules are the actual source of the luminescence." On shaking, the "globes" completely disintegrate, leaving a suspension of granules. This description recalls

the granules in earthworms and in *Heteroteuthis*, except that *Heteroteuthis* granules occur in clumps without the sheath which surrounds the "globes."

When the light is extinguished by adding distilled water, the globes were observed to swell, liberating groups of granules. The granules themselves did not swell. In a series of experiments with various solutions, Hickling found that luminescence was most prolonged in pure NaCl, KCl, KNO_3 , MgSO_4 , and cane sugar solutions about isotonic with sea water. Calcium was not necessary for luminescence. Alcohol and glycerine would not support luminescence in any concentration, probably because of their ready penetration. Copper sulfate solution extinguished the light immediately. A certain range of H-ion concentration was necessary for luminescence and the suspension of granules in sea water became progressively more acid (apparently due to CO_2), as time went on and the light-intensity gradually decreased. The previously observed necessity for abundant oxygen was confirmed.

It is apparent that the behavior of these granules is much like luminous bacteria. When filtered, the filtrate showed only slight luminosity and no luciferin-luciferae reaction could be demonstrated with filtrates. Their form never disappeared, i.e., they never dissolved completely. When cytolytic agents, like saponin or sodium glycocholate were added, no decrease in light intensity occurred, and no swelling of the granules could be detected.

The light was dimmed but not extinguished at 38° but went out rapidly at 47° with some reversal in cooling. Heated to 55° , the extinction was permanent. Hickling gave some experiments to indicate that sunlight decreased the luminescence, but this effect may have been partly due to heating.

All the above characteristics are such as would be shown by luminous bacteria, and Haneda (1938) has in fact cultured bacteria taken from the gland itself, using special precautions to guard against contamination by ordinary luminous bacteria. In the gland the bacteria are found in sacks, which hang grape-like from the gland cells, or free in the lumen of the gland when a sack (equivalent of Hickling's globe) breaks. They never occur within the gland cells. In pure culture the bacteria are short rods, 2.2 to 3.6μ long and 1.0 to 1.3μ broad. They are gram negative, have no capsules, and no spore formation was observed. Specific agglutination reactions with rabbit sera were obtained.

Haneda photographed the spectrum of the bacterial light through a quartz prism and measured the blackening of the plate with a microphotometer. The emitted light extended from 638 to $430 \text{ m}\mu$, with



FIG. 169. *Physiculus japonicus*. Left, side view. Right, ventral view with insert showing opening of luminous gland, S; anus, A; and urogenital opening, o. After Kishitani.

great blackening between 579 and 459 $m\mu$ and maximum blackening of the plate at 510 $m\mu$.

Anacanthini, Gadidae. The Gadidae stand next to the Macrouridae in relationship. At least two species of *Physiculus* (Fig. 169) contain luminous organs with luminous bacteria. Hickling (1931) had sug-

gested that the gland of *Physiculus*, shown in Fig. 170, was homologous to that of *Malacocephalus* without knowledge of the work of Kishitani (1930), who demonstrated the presence of the symbiotic bacterium, *Micrococcus Physiculus* in the gland of thirty different individuals of *P. japonicus* from the Gulf of Mutsu, Japan. The symbiotic form had definite cultural characteristics and gave specific agglutination reactions quite different from an ordinary salt water bacterium. *Microspira asamushiensis*, found on the skin and in the alimentary canal of the fish.



FIG. 170. Section of the luminous gland of *Physiculus japonicus*. Left, longitudinal section; right, transverse section. After Kishitani.

Observation of living *Physiculus* has been made by Haneda (1938), who was able to see the light from the ventral organ shining through the disc in two out of ten fishes. One of the two fishes was light in color and the whole of its under surface was luminescent. The fishermen of Asamushi know of the luminescence of this fish, which they call "Donko." When they are hauled up in nets from the bottom in a luminous condition the fishermen say "Donko has yawned." In an unpublished manuscript, Haneda has listed *Lotella physis* of the Gadidae as luminous.

Bereycomorphi, Monocentridae. Apparently the first recorded observation of light production in this family is due to Stead who described in *Fishes of Australia*, published in 1906, "peculiar luminous disks" near the mouth of *Monocentris gloria maris*. During a visit to Japan

in 1916 the author (1917) saw the two light organs of the knight-fish, *Monocentris japonica*, one on each side of the under surface of the tip of the lower jaw, as shown in Fig. 171. "The light is a weak glow with the faintest bluish tinge and is continuous and of almost unvarying intensity." Attempts were made to demonstrate a luciferin-luciferase reaction without success. After the discovery of luminous bacteria in the fishes *Anomalops* and *Photoblepharon*, the author (1923) predicted that the luminous organs of *Monocentris* would be found to contain luminous bacteria also, a prediction confirmed by Yasaki (1928).



FIG. 171. The knight-fish, *Monocentris japonica*. Left, side view. Right, ventral view of lower jaw containing a light organ in the black region near tip. After Yasaki.

Before Yasaki's work, *Monocentris* had been studied by Okada (1926), who worked out the morphology and histology of the glands and made observations on the living fish but missed the bacteria. *Monocentris* can produce light day and night but is not continuously luminescent for twenty-four hours. There are periods, particularly during the day, when no light is visible. Then the light will spontaneously appear, and if the water is agitated, continue for several hours. When ammonia is added or when placed in fresh water, where they live for ten hours but are definitely uncomfortable, a bright light is easily observed. Yasaki has noted that light can be seen within the mouth if examined at those times when no light is showing from the outside. Okada was unable to demonstrate nerves entering the gland, and the whole behavior, particularly the spontaneous luminescence, suggests chromatophore control. It would be most interesting to test the effect of adrenaline on the luminescence of this fish.

Okada found that "By rubbing the luminous protuberances with a piece of stick or by scraping them with a knife edge, the luminosity

could partially be transferred to the surface of the stick or knife remaining visible there for several seconds . . . no luminous material was seen excreted into the water by a living fish; the luminescence is 'extracellular' but 'intraglandular.' " This condition is easily understood by the structure of the organ, which is made up of secretory tubules, a lumen, and a number of ducts to the outside, somewhat similar to the organ of *Photoblepharon* and *Anomalops*. Okada found the granular secretion in the tubules readily stainable with hematoxylin and considered that the tubule epithelium broke down to form the secretion. He regarded the granules, not as bacteria, but as a "metamorphosis of the cytoplasm at the time of cellular destruction."

In demonstrating the bacterial nature of the light of *Monocentris*, Yasaki examined 79 fish microscopically and grew cultures of the bacteria obtained from the organ under sterile conditions. With dark field illumination the *Vibrio* type organisms are clearly visible in rapid motion. They are 1.5 to 3 μ long by 0.5 μ wide, gram negative, with cultural characteristics different from ordinary bacteria, and with specific agglutination precipitation and complement fixation reactions. When injected into shrimp, fishes, frogs, mice, rabbits, and guinea pigs, there is no evidence of virulence, nor do they render the animal luminous, thus behaving differently from the pathogenic *Microspira phosphoreum* which Yasaki (1927) had found to be the cause of a luminous disease of the fresh water shrimp of Lake Suwa.

Okada carried out regeneration experiments with the knight-fish which should be attempted with other luminous animals, as too little is known regarding regeneration of luminous organs. He excised the photogenic organs of eight fishes in varying extent. The wound healed quickly and the integument was completely regenerated in two weeks. In serial sections it was observed that the tubules, whether entirely or partly removed, did not regenerate but were replaced by spongy tissue with very large blood vessels. The granular matter in the remaining tubules did not increase in amount, one result which led Okada to the conclusion that the granules were not bacteria.

Bercycomorphi, Anomalopidae. This family contains three genera, *Photoblepharon*, *Kryptophaneron*, and *Anomalops*, each with a single species. *Photoblepharon* had been previously called *Sparus* and also *Heterophthalmus*, and *Anomalops* was originally called *Heterophthalmus* also. *Kryptophaneron alfredi* is known only from an individual found floating at the sea surface off the north coast of Jamaica, British West Indies. Its light organ, similar to that of *Photoblepharon*, was described by Dahlgren (1908), and the fish was placed in a new genus by Sylvester and Fowler (1926). Nothing is known of its habits, but the

luminescence is undoubtedly similar to that of the other two genera.

Photoblepharon and Anomalops constitute a pair of the most unusual luminous animals. Both are rather rare but have been known for a long time. Photoblepharon palpebratus, the "ikan leweri batu" of the natives, is restricted to Amboina and the Banda Islands of the East Indies, while Anomalops katoptron, or "ikan leweri laut," is found not only in the East Indian region but in South Sea islands as well—the New Hebrides, Fiji, Paumotu, and Raratonga. Both are surface fish. Photoblepharon swimming singly or a few together among the coral while Anomalops may swim in schools of a hundred or more in somewhat deeper water.



FIG. 172. Luminous fishes of the Banda Islands. Two Photoblepharon (left) and three Anomalops (right), from an original photograph. Note that one Photoblepharon has its light organ covered.

The earlier observers with preserved material at their disposal were ignorant of the purposes of the large conspicuous organs (shown in Fig. 172) immediately under the eye. Boddaert in 1781 thought the function was to shield the eyes of the fish from injury by the branches of coral among which it lived, while Lacépède in 1803 considered it a protection of some sensitive tissue against the rays of the tropical sun. Günther (1880) first suggested that the structures were light organs, while Vordermann (1900) actually observed the living fish in 1897 and saw the light. The short paper of Vordermann and the description recorded in the narrative of the *Siboga* expedition by Weber (1902) contained all our knowledge of these fishes until the appearance of an extensive paper by Steche (1907, 09). This excellent monograph deals

largely with the histology of the luminous organ and also contains a few physiological observations. Thus Steche determined that the light shone day and night continuously with an intensity (for one organ of Photoblepharon) of 0.0024 meter-candles. Mechanical pressure or chemical stimulation is quite unable to increase the intensity of the light, which can be "turned off" completely only by mechanical contrivances connected with the organ.

The author (1921, 23) visited the Banda Islands in 1920 where both Photoblepharon and Anomalops are fairly common, and continued studies of the fish, coming to the conclusion that the light was due to luminous bacteria growing symbiotically in the organ. Recently Haneda (1943) in a paper in Japanese, has doubted the bacterial origin of the light of Anomalops, chiefly on the ground that he was unable to grow luminous cultures of the organism (but did obtain some non-luminous growths), although the symbiotic luminous bacteria from other fish, *Monocentris*, *Physiculus*, *Acropoma*, *Leiognathus*, and *Malacocephalus* are easy to cultivate. In addition he found that the cells of the organ were different from those in the above fish and that the organ is closed, i.e., it has no duct to the exterior.⁶ According to Steche (1909) the organs of both Photoblepharon and Anomalops do have pores leading to the outside, a point confirmed by Dahlgren. The author feels very sure of the bacterial origin of the light which will be discussed later.

Morphology. In both fishes, the luminous organ is a compact mass of white to cream-colored tissue, flattened oval in shape, lying in a depression just under the eye and in front of the gills. The organ is well suited for experimentation, as it is attached only at the dorso-anterior end and can be cut out with the greatest ease, giving a piece of practically pure luminous tissue. The back of the organ is covered with a layer of black pigment which serves to keep the light from shining into the tissues of the fish. In both genera there is a mechanism for obscuring the light, but curiously enough the mechanism developed is totally different in the two, notwithstanding the fact that in structure the organs are identical and in every detail except proportions the fish are very similar.

In *Anomalops*, by means of the hinge at the antero dorsal edge, the organ can be turned downward until the light surface comes in contact with black pigmented tissue forming a sort of pocket. The light is thus cut off. In *Photoblepharon*, a fold of black tissue has been developed on the ventral edge of the organ socket, which can be drawn up over

⁶Haneda has written me that he examined only three small specimens at Mamukwari, Dutch New Guinea, and would like to make a further examination.

the light surface like an eyelid, thus extinguishing the light. Why these two fish, so similar in most respects, and especially in the general structure of the luminous organ, should have developed such totally different means of extinguishing the light is a mystery.

Although luminescence is continuous in the organ, in the sea the light of *Anomalops* is constantly being turned on and off, according to Steche, ten seconds light and five seconds dark. Photoblepharon in its natural environment shows its light nearly continuously, but in glass jars, either as a result of partial asphyxiation or excitement, its light is also intermittent. The animals appear to be swimming about with bright flashlights which are turned on periodically. According to the natives, the organ is used as a searchlight. Steche also considered it a searchlight to attract prey and the flashing as a means to mislead its prey.

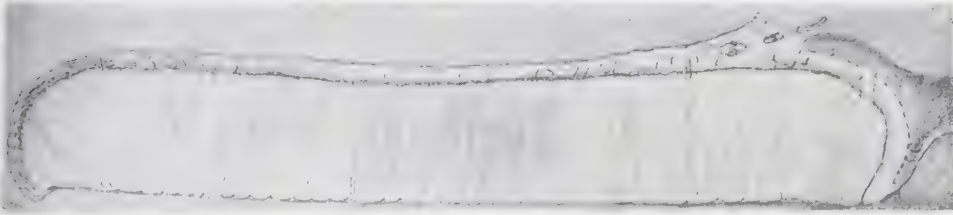


FIG. 173. A cross section of the luminous organ of *Photoblepharon*, showing the tubes containing luminous bacteria. A rich supply of blood capillaries runs between the tubes. After Steche.

When brought into the laboratory, in small glass jars, where the supply of air is limited, the fish eventually lose control over the closing mechanism. Just before death from asphyxiation, when the fish swim slowly upside down, the light is usually not visible, but when movement ceases, the organ is exposed and gives forth light, both in *Anomalops* and *Photoblepharon*. This presumably means that the muscles involving the closing mechanism pass into the contracted condition on death. The relaxed condition of these muscles, then, would correspond to the closed position of the organ.

Histology. Steche described the organ as an acinose gland made up of a large number of gland tubes parallel to each other and extending completely across the organ, from the back pigmented surface to front transparent surface, where the light emerges, as shown in Fig. 173. In a parallel section of the fresh organ it is very easy to see these tubes and also the blood vessels which run between them. A cross section of the tubes shows that they are pressed into a polygonal shape, separated from one another by sparse connective tissue and arranged in a ring about a blood vessel, from which they receive oxygen and

food supply. The back ends of these tubes meet a layer of cells containing small granules of guanin, which are believed to act as a reflecting layer, in analogy with such a layer found in the luminous organs of other fish.

Just below the front surface of the organ a number of tubes unite to form a common reservoir which connects with the exterior by a pore (20 to 30 μ wide) passing through cutis and epidermis. The pores are scattered over the surface of the organ and were overlooked in Steche's first description of the tissue.

No luminous material is passed out of the gland, a point confirmed by the author from observations of living fish, so that Steche believed that the luminous substance was burned in the reservoir at the front surface of the gland. The luminescence would therefore be extracellular but intraglandular.

At the front end of the gland tubes there is a 1- to 2-layered epithelium which passes at the pores into the epithelium of the outer surface of the organ. Under the epithelium is a tough connective tissue in which are embedded blood vessels and nerves. The red blood vessels are very clearly visible in the living organ outlined against the white of the gland. They arise as nine to thirteen vessels passing from both the lower and upper edges of the front surface of the gland and branch to smaller vessels meeting near the middle. A peculiar valve occurs where branch vessels leave the main artery. The blood supply is exceedingly rich. Steche could not determine the point of ending for the nerves which follow the blood vessels, but thought they pass to the gland-tubes and control the secretion.

Bacteriology. The fresh gland is firm enough to be cut with a razor into fairly thin sections, in which the parallel gland tubes with blood vessels running between them can be clearly seen under the microscope. On application of pressure to the cover-glass, the gland tube contents flow out and mingle with the sea water bathing them, forming a white, milky emulsion. In this emulsion there are a great number of small granules and rods, often arranged in spirillum-like rows. The rods are unquestionably bacteria, as they can be seen to move of their own accord, often with a corkscrew-like motion.

The gland itself when excised retains its ability to light for a long time, and the fishermen of Banda make use of the light organ as bait in fishing at night. A suspension of the organ material in sea water will retain its luminescence for about eight hours at 29° if concentrated, and a shorter time if more dilute. This is quite in accord with the behavior of luminous bacterial suspensions at these high temperatures. Stained smears of the gland show the bacteria clearly

and in great abundance. Various additional facts in favor of the bacterial origin of the luminescence are:

1. The light organ is extraordinarily well supplied with blood vessels and the emulsion fully as sensitive to lack of oxygen as are luminous bacteria. Light ceases very quickly in absence of oxygen.
2. If dried, the organ will give only a faint light when again moistened with water. This is characteristic of luminous bacteria. The luminous organs of most other forms can be dried without much loss of photogenic power.
3. Luciferin and luciferase cannot be demonstrated.
4. The light is extinguished without a preliminary flash by fresh water and other cytolytic (bacteriolytic) agents.
5. Sodium fluoride of 1 to 0.5% concentration extinguishes readily the light of an emulsion of the gland.
6. Potassium cyanide has an inhibitive effect on light production in about the same concentration as with luminous bacteria.

Actual proof that the bacteria found in the organ are luminous can only come when these are grown artificially. All attempts in this direction have failed. The author obtained good growths of bacteria on peptone-agar, but they produced no light. However, conditions for bacteriological work at Banda were poor. If this symbiotic form requires rather special food material to produce light, it is, perhaps, not surprising that culture experiments failed.

Percomorphi, Acropomatidae. In the southern part of Japan there occurs a luminous fish, *Acropoma japonicum*, the "hotaru-dyako" of the fishermen, whose whole under-belly emits a continuous bluish white light. The luminescence has been studied by Yasaki and Haneda (1936) and Haneda (1950). Close inspection shows that the diffuse light comes from a definite U-shaped organ in the muscle tissue, not visible from the outside, as shown in the diagram in Fig. 174. It is made up of lens, reflector, light gland, and a long duct opening near the anus. The reflector is whitish in color and the "lens" is modified muscle tissue, partially transparent and opalescent. The function of these structures is to throw the light from the gland cells proper over a wide region of the belly of the fish, thus constituting an "indirect" type of light organ.

The duct lies between the sides of the U which is made up of gland cells forming compartments in which luminous bacteria are found. These bacteria are motile, of Coccus or short rod form, with definite cultural characteristics. They are easily grown and have been named *Coccobacillus acropoma*.

Although only one species of *Acropoma* is recognized in Japan, Haneda (1950) has observed that the light organ varies in shape and position in two types of fish, which may represent different species.

Percomorphi, Leiognathidae. Other luminous fish of the same general type as *Acropoma* belong to the Leiognathidae. One, shown in Fig. 175, was described as a species of *Equula* (now called *Leiognathus*), caught on the south coast of Java and near other islands, where it is

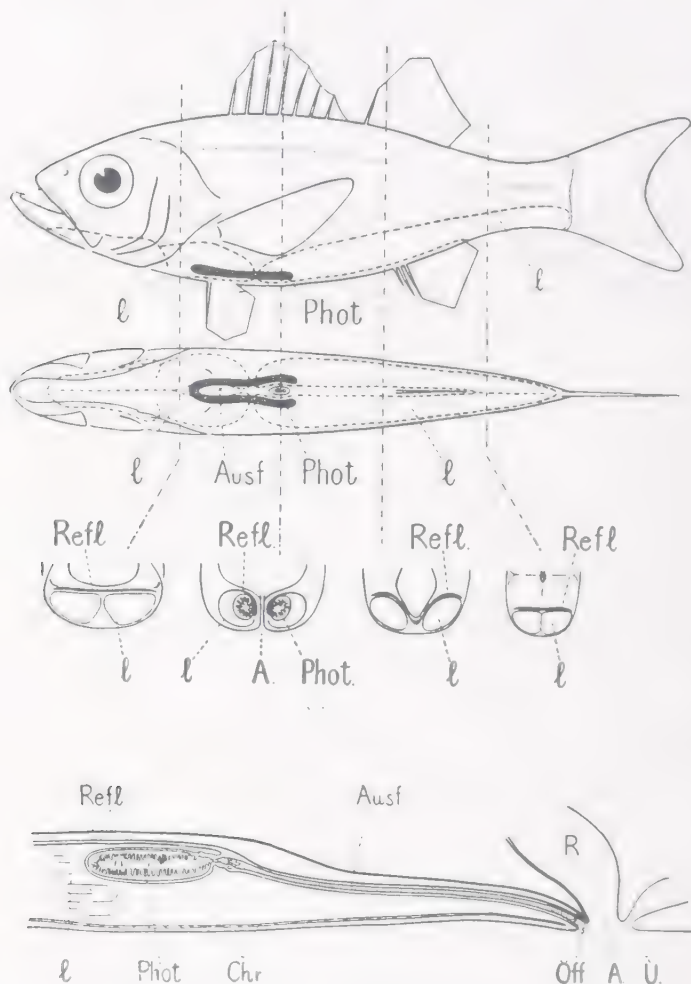


FIG. 174. *Acropoma japonicum*. Above, the fish from the side and from below with cross sections at the levels indicated by dotted lines. Below, a longitudinal section of the photogenic organ (phot) and its duct (Ausf); l., lens; Refl., reflector; R., rectum; A., anus; U., urogenital opening; Off., opening of luminous gland. After Yasaki and Haneda.

called "ikan pepetik" or "shan shan" by natives who are well aware of its luminosity. It is caught in large quantities, dried, and eaten with rice. Harms (1928) observed the living fish and studied the morphology and histology of the light organ. The light is bluish green and very strong in the region of the gill opening. It comes from a flattened ring of tissue which surrounds the esophagus where it joins the stomach,

but the light is scattered so that the whole belly wall appears luminous. When the fish is opened the light organ is readily visible in the ventral part of the swim bladder cavity. When excised the light continues for days. Another species, *Gazza minuta*, with a diagram of the light organ, is shown in Figs. 176 and 177.

The organ is made up of finger like glands more or less parallel to each other and radially arranged around the esophagus. Several such glands open into a short duct to a reservoir from which further ducts connect with the esophagus between the mucous villi. The contents of the ducts are made up of rod-shaped bodies which Harms designated

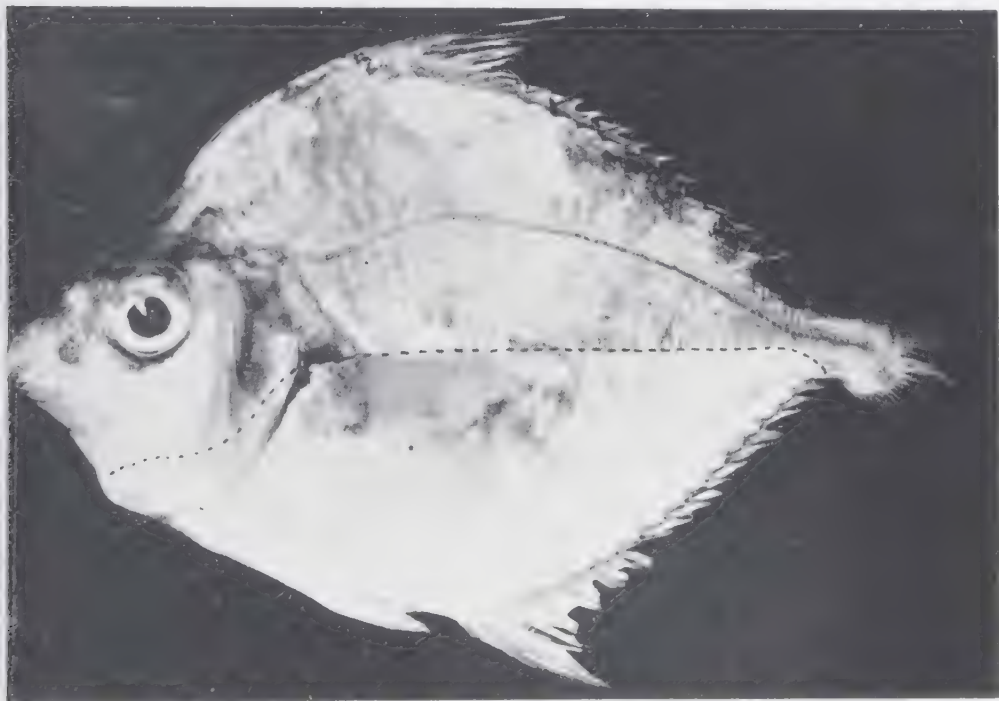


FIG. 175. *Leiognathus equula*. Photo by Y. Haneda.

as luminous bacteria from staining reactions although he did not attempt culture experiments. There is a sort of reflector, represented by a thick layer of tissue next to the esophagus, and a so-called lens. The light organ is well supplied with blood vessels but not nerves and in many respects resembles the light organs of the *Anomalopidae*.

In 1938 Haneda began study of the luminescence of members of *Leiognathidae*, *Leiognathus equulus*, *L. fasciatus*, *L. lineolatus*, *L. splendens*, *L. dura*, *L. bindus*, *Gazza minuta*, *Secutor insidiator*, and *S. ruconius*. All are shallow water fish and all have a luminous organ of the indirect type, like that of *Acropoma*. Haneda's (1940) description is essentially like that of Harms, and he was able to isolate and cultivate

the bacteria, naming them *Coccobacillus equule*. The bacterial light arising in the esophageal ring of glandular tissue, is projected to the surface of a bright reflector which lines the body cavity, and is then reflected to the sides and ventrally through a system of lenticular muscles. It is visible externally as a diffused greenish blue light, sometimes intermittent. Control appears to be by chromatophores as it is necessary to handle the fish or remove it from the water before the luminescence is displayed. The gland opens into the esophagus by two ducts, and it is through these that the luminous bacteria gain entrance. In a later paper, Haneda (1950) described luminescence from observation of

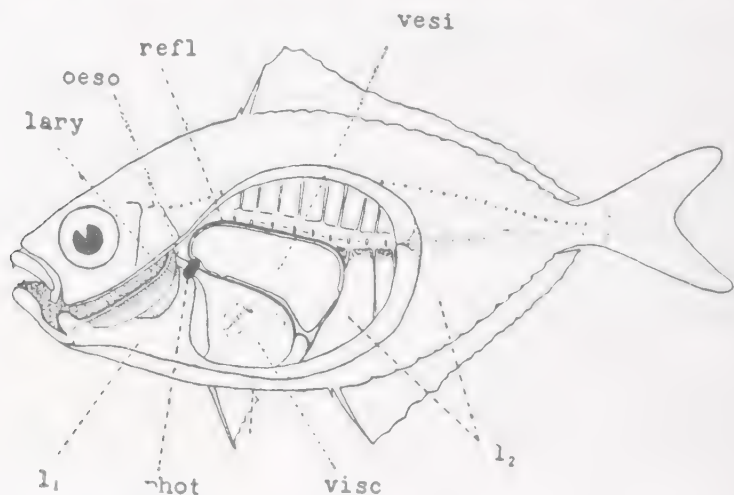


FIG. 176. Section of light organ of *Gazza minuta*, showing luminous gland, phot; reflector, refl; lens, l_1 , l_2 ; larvnx, lary; esophagus, oeso; internal organs, visc; air bladder, vesi. After Y. Haneda.

fifteen species of *Leiognathidae*, distributed in three genera (*Leiognathus*, *Secutor*, and *Gazza*).

Percomorphi, Serranidae. The only member of this family now known to luminesce is *Apogon marginatus*, found in shallow seas of southern Japan and described by Kato (1947) in a Japanese paper whose abstract is as follows: "The organs are located at three distinct points of the intestine in small diverticula, one at the proximal and two at the distal end. The wall of these diverticula consists of photogenous cells, each of which is elongate in shape, striated at the free border, with a nucleus at the base and filled with an eosinophile secretion. Photogenesis appear to be intracellular. This is the first instance of an automatic luminous organ which is located in the intestinal wall." It is not definitely known whether the light is due to luminous bacteria.

Pediculati. There are a number of closely allied families of fish, frequently characterized by the use of lures in attracting their food

and collectively called angler-fishes. That the filamental lure or illicium, a modification of the first dorsal fin ray, is actually used for its presumed purpose is attested by a number of observations collected by Gill (1909) on the behavior of shore species in daytime. It is not surprising to find that female deep sea angler-fish have the bulbous end of the organ, the esca or bait, developed into a luminous organ.

Another peculiarity of the deep sea forms belonging to Ceratioidea, is the existence of males parasitic on the females. In fact, until the twenties of this century the males had been assigned to a separate

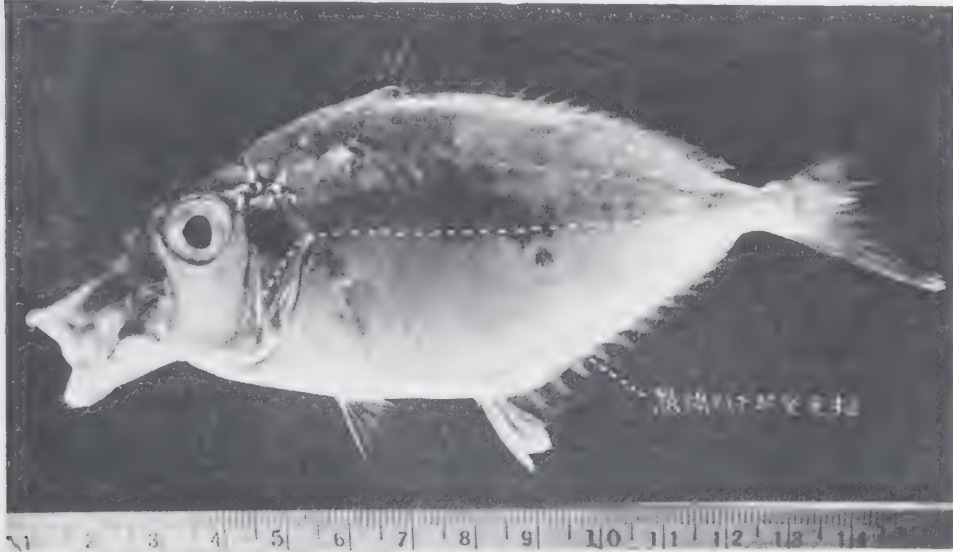


FIG. 177. *Gazza minuta*. Photo by Y. Haneda.

family, the Aceratiidae where females had never been found, while the females were described under a number of families of the ceratioid group. Regan discovered the relationship in 1925, after finding that an aceratiid was a male with its snout actually grown into the skin of a female ceratiid. The males of all Ceratioidea, free-swimming or parasitic on females, have no luminous lure but only remnants of an illicium embedded in the skin, a situation which led Parr (1930) to suggest that the light of the female is used to attract the male.

However, the use as a lure for food is striking, since the illicium of some species can be extended and retracted by a complicated muscle system. Bertelsen (1943) has described the mechanism in *Ceratias holboelli*, a large angler-fish, around 100 cm in length. The esca may be protruded 30 cm in front and withdrawn to a position immediately above the mouth, suggesting that unwary prey may be lured by the luminous bait straight into the jaws of its captor.

The shore forms of angler-fish have been known since classic times as marine frogs or fishing frogs. *Lophius piscatorius* or related forms were described by Rondelet, Belon, Salviani, Aldrovandi, and others. Their deep sea relatives appear to have received first attention in 1837 when Reinhardt described a specimen found in Greenland as *Himantolophius groenlandicus*. Others were obtained from time to time but were extremely rare until the cruise of the *Challenger* and ships of other oceanic explorations. The various Danish expeditions on the *Dana* have been particularly successful. The specimens have been described by Regan (1926) and Regan and Trewavas (1932). According to Waterman (1939), who has made special studies on these forms, 860 specimens are now recorded in the literature, representing 41 genera and 11 families. Frequently a single specimen has established a genus or a family. Much of the systematic work has been pioneered by Regan (1912, 25, 32) while one of the most recent accounts is that of Koefoed (1944).

Willemoes-Suhm (1875) wrote of seeing luminescence in the tip of the head barbel of a deep sea lophioid fish, but few others have also had the opportunity. Beebe (1934) observed the lemon-colored light of the bulb of the illicium of an angler fish from the bathysphere and has also seen the luminescence of *Melanocetus ferox*, *Himantolophus azurlucens*, *Borophryne apogon*, and *Linophryne arcturi*. The light from the bulb of *Borophryne apogon* was described as purplish, while the behavior of *Linophryne arcturi* (Beebe and Crane, 1947) was as follows: "The white base of the candle-like organ showed distinct luminescence in the dark room during the first three minutes after capture. In addition, all the larger fangs were dimly outlined with luminescence, apparently resulting from a mucous coating." Some deep sea angler-fish are shown in Fig. 178.

Waterman (1939) observed a living *Dolopichthys* taken in the Sargasso Sea and quickly placed in cold sea water where it lived for ten minutes. The esca light organ "remained dark until the fish was quite actively stimulated mechanically. When it did luminesce, the light was confined within the esca, which was transparent and more or less free from pigment distally, and no trace of luminous secretion was observed to be extruded from the external pore of the organ. The light was bluish green in color like that of the majority of luminous organisms and lasted for five or six seconds beginning dimly and rising to a peak in a second or so, maintaining the maximum intensity for several seconds, and then slowly fading out." This behavior suggests a hormone control of the luminescence. The author (1931) observed no luminescence on injecting adrenaline into a moribund *Linophryne*.

arborifer, but further studies of adrenaline should be made at the first opportunity.

Classification. The Pediculati are divided into 3 suborders and 16 families according to the classification of C. T. Regan. In the following



FIG. 178. Various kinds of deep sea ceratioid fish. 7, *Ceratias couesi*; 5 above, *Melanocetus pelagicus*; 6, *Oneirodes niger*; 5, below, *Melanocetus johnsoni*, after Brauer. A, *Himantolophus azurlucens*. After Beebe and Crane.

list, genera with presumed luminous organs are italicized. Since the females of all Ceratioidea possess illicia with an esca, they are probably all luminous. The genera with an asterisk are from males with no illicium.

Lophioidae

Lophiidae (Lophius, Lophiomus, Stadenia, Chirolophius)

Antennarioidea

Antennariidae (Antennarius, Hystrio, Pterophryne, Saccarius, Tetrabranchium)

Brachionichthyidae (Branchionichthys)

Chaunacidae (Chaunax)

Ogcocephalidae or Malthidae² (Malthopsis, Halicometus, Ogcocephalus, Dibranchius, Dibranchichthys, Coelophrys, Halieutacea, Halieutopsis, Dibranchopsis)

Ceratioidea

Melanocetidae (Melanocetus, Centroctetus,* Xenoceratias*)

Diceratiidae (Diceratias, Paraneirodes, Caranactis*)

Himantolophidae (Himantolophus or Aegaeonichthys, Lipactis,* Rhynchoceratias*)

Oneirodidae (Oneirodes, Dolopichthys or Monoceratias, Chirophryne, Ctenochirichthys, Lophodolus, Tyrannophryne, Centrophryne, Chaenophryne, Lasiognathus, Thaumatchthys, Amacrodon, Trematorhynchus*)

Laevoceratiidae (Teleotrema,* Laevoceratias*)

Gigantactidae (Gigantactis, Rhynchactis)

Neoceratiidae (Neoceratias*)

Ceratiidae (Ceratias, Cryptosparus, Mancalias, or Myopsaris)

Caulophrynidae (Caulophryne, Ceratocaulophryne)

Photocorynidae (Photocorynus)

Linophrynidae (Edriolynchus, Cryptolynchus, Acentrophryne, Borophryne, Linophryne, Haplophryne,* Anomalophryne,* Nannoceratias,* Aceratias*)

Histology. The earliest work on histology of the light organs of Pediculati is by von Lendenfeld (1905) who described a "tubular radiating organ" in the retractable median papilla on the forehead (near mouth) of *Malthopsis spinuloza*, one of the Ogcocephalidae. The lumen of the papilla is lined with a simple epithelial layer of cylindrical cells, presumably photogenic in character.

On the margin of the body of *Malthopsis*, there occur bud-like organs which Trojan (1905) has studied and described in an appendix to the von Lendenfeld (1905) paper. Trojan came to the conclusion that the body papillae were either radiating or sensory but since they had a large nerve supply he rather favored the theory they were hydrostatic pressure receptors.

Extensive work on the structure of the light organs of the Pediculati is due to Brauer (1908), who studied and described five species: *Gigantactis vanthoeffeni*, *Ceratias couesi* and *Halicometus ruber*, *Oneirodes* (*Dolopichthys*) *niger*, and *Chaunax pictus*.

These forms all possess an illicium with the esca light organ at the end, and certain of the Ceratiidae also have caruncles, pear- or club-shaped organs at the beginning of the dorsal fin. The tentacle organ

These genera are all included in the list of luminous fish published by Brauer (1927).

and the caruncle have essentially the same structure, a spherical gland with a lumen, surrounded by a reflector and pigment mantle. Connective tissue septa extend inward from the walls dividing the gland into compartments lined with cylindrical or cubic epithelial cells, which appear to disintegrate into the finely granular secretion. The lumen opens into a second more distal cavity which communicates with the outside by a short duct opening in the mid dorsal line of the tentacle swelling. There is a rich blood vessel supply to the gland but no nerves (except those to sensory papillae of the esca) and no definite muscles

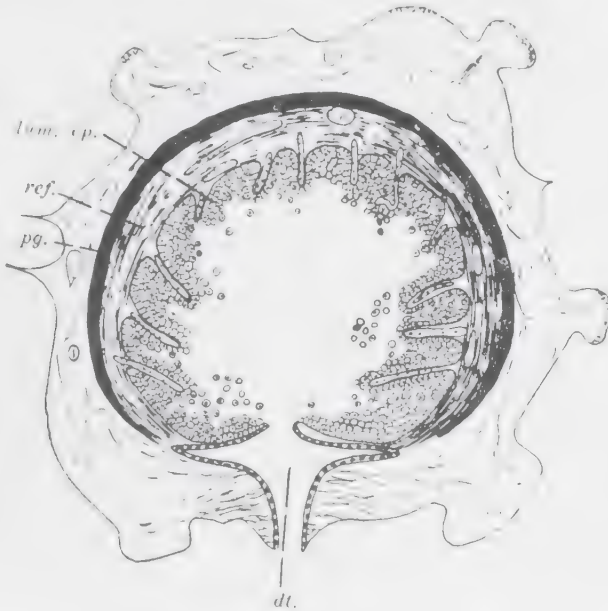


FIG. 179. Section of the light organ in the esca of *Gigantactis*, showing luminous epithelium, lum. ep.; reflector, ref.; pigment layer, pg.; opening, dt. After Brauer.

which might function to empty the contents. The structure of the tentacle organ of *Gigantactis* is shown in Fig. 179.

In *Chaunax* the tentacle is very short and the light organ is unpigmented, made up of many glandular tubes with a single layer of epithelial cells. It is not possible to demonstrate openings to the outside in this form.

Structure of the tentacle organ of *Gigantactis* as described by Brauer, is very much like that of fishes known to harbor luminous bacteria and Dahlgren (1928), from histological study of the esca organ of a species of *Ceratias* came to the conclusion that the granular bodies in the lumen were bacteria. It would be most important to confirm this belief with bacteriological studies of living fish.

Lyomeri, Saccopharyngidae. Deep sea eels of the family Sacco-

pharyngidae appear to possess luminous organs. One case was described by Brauer (1908), who considered that a swelling, pigmented above but colorless below, at the end of the tail of *Macropharynx longicaudatus* was photogenic. According to Rauther (1927), *Zugmura* considered this fish to be a young form of *Gastrostomus bairdi*, which lacks light organs. The other case is due to Beebe (1932), who has figured the tail-light organ of *Saccopharynx harrisoni* caught at 900 fathoms, south of Bermuda, and reported a number of other luminous



FIG. 180. *Saccopharynx harrisoni*, showing luminous troughs and caudal organ at tip of tail. After Beebe.

regions. The fish is shown in Fig. 180. After describing two troughs of whitish material which extend along the back from head to within 10 cm of the tail tip and a number of scarlet papillae, Beebe wrote: "At an equal distance beyond these [papillae] begins a most amazing luminous organ, a leaf-like, compressed, almost transparent zone, traversed with a network of large blood vessels. Posteriorly the dorsal and ventral tips are prolonged into exaggerated, finger-like imitations of the preceding papillae. These are scarlet pigmented, not pink with the blood like the rest of the organ. A central longitudinal band of scarlet-dotted purplish black divides the organ into two. The dorsal finger is much the longer and freer, and there are two more of these dorsal structures trisecting the remainder of the tail. Beyond the last

organ the tail rapidly diminishes in size, and shows considerable scarlet and purple arranged along both profiles, the scarlet dominant from the last finger-papilla to the tip. . . .

"In addition to the faint colorless glow from the nuchal troughs, we distinctly saw a faint pinkish glow and twice a flash from the specialized caudal organ, and also from several of the scarlet papillae. Under the lens I could later see blood corpuscles moving very slowly along the exposed veins."

Fish with Skin Photophores

The fish in Group II, with photophores, belong to the orders, Isospondyli, Iniomi, and Haplodoci. The first two orders are usually considered to contain the true deep sea fish, the stomiatoids and the scopoloids or myciophoids. Only one genus of the Haplodoci, *Porichthys*



FIG. 181. *Porichthys notatus*, showing the photophores. After Greene and Greene.

thys of the *Batrachoididae* is luminous. This genus contains the California toad-fish, usually classified as a surface form. It thus presents an exception to the general statement that fish with photophores are from the deep sea. However, *Porichthys* is caught at the surface only when it comes inshore to breed; at other seasons it returns to deeper water, but the exact habitat is unknown.

Haplodoci, *Batrachoididae*. The *Batrachoididae* include not only luminous toad fishes but a number of non-luminous forms, including the Atlantic toad-fish, *Opsanus tau*. *Porichthys notatus*, the California form is best known as the first animal in which hormone control of luminescence has been demonstrated.

An intimation that fish of the genus *Porichthys* might luminesce, appeared in a paper by Solger (1881), published at a time when the work of Ussow (1879) and Leydig (1879, 81) on the use of the pearly spots of fish was under discussion. Solger published a figure of a

section of one of the spots of *P. porosissimus*, designating it as a light organ. This species has now been separated (Hubbs and Schultz, 1939) as a new genus, *Nautopaedium porosissimus*. *Porichthys porosus*, a non-luminous species, has been separated as *Aphos porosus*, leaving five or six species in the old group, *Porichthys*, all luminous and all restricted to the Pacific Coast from Alaska to Colombia.

Test (1889) and Eigenmann and Eigenmann (1889) first called attention to the structure of the phosphorescent spots in *P. margaritatus*.

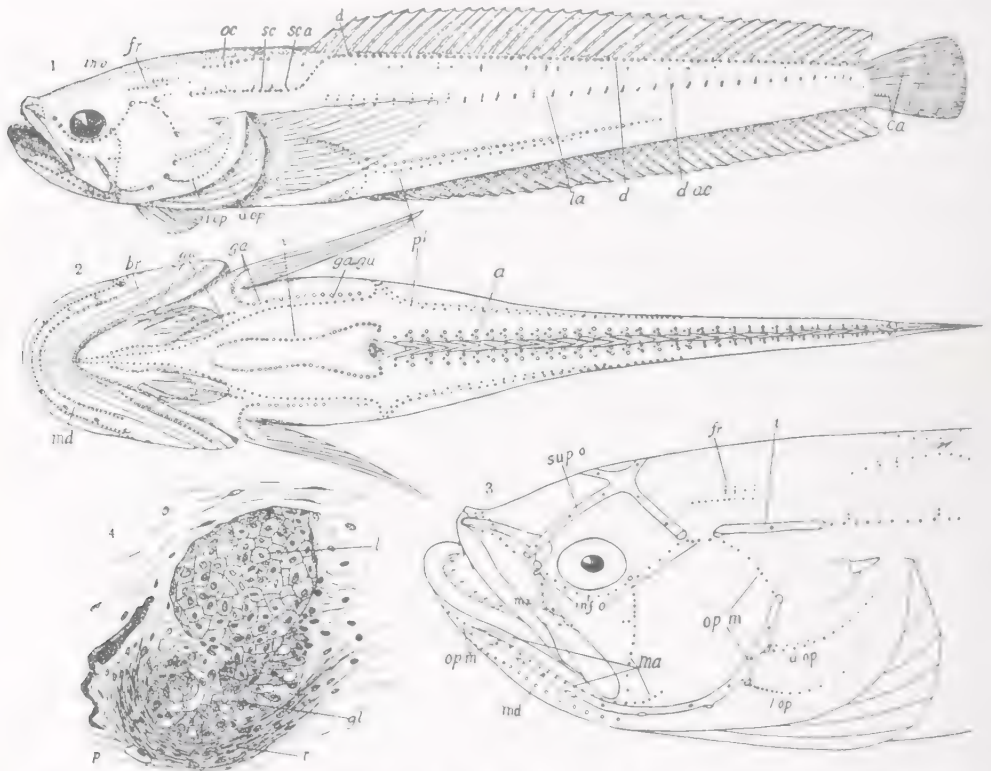


FIG. 182. Diagram of photophore distribution of *Porichthys notatus*. At lower left a cross section of a photophore. After Greene.

often called the midshipman, because of the shining pores on its skin supposed to resemble the buttons on a middy's jacket. Extensive knowledge of the histology, embryology, and physiology of the light organs of *P. notatus* comes from the work of Greene (1899) and Greene and Greene (1924). This species, shown in Fig. 181, is sometimes called the California singing fish because of the nocturnal humming sounds which are produced by vibration of the air bladder. The fish lives in deep water most of the year and comes toward shore in spring and early summer to breed, a habit which led Hubbs (1820) to believe that luminescence might be associated with the breeding season.

As many as 840 luminous organs have been counted on *Porichthys notatus*, some one hundred of which are rudimentary. They are rather small and have sometimes been mistaken for lateral line organs, as they often follow the pattern of the lateral line system. Their complicated distribution is shown in Fig. 182.

Histology. The light organs of *P. notatus* are deeply embedded in the dermis, a mass of photogenic cells surrounded by a reflector and a layer of melanophores. The light passes downward through a lens and corneal-like epidermal covering. No nerves could be demonstrated by Greene, but there is a rich supply of blood vessels. Fig. 183 shows the structure of a typical photophore. The reflector is extraordinarily

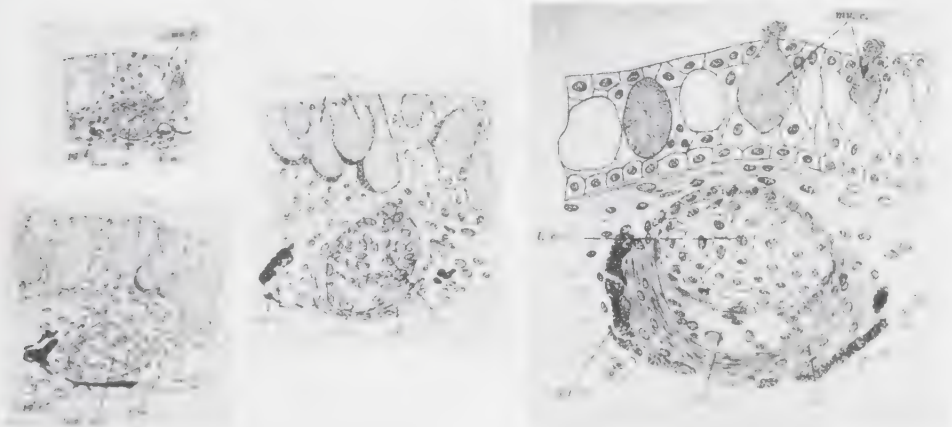


FIG. 183. Four stages in the development of a photophore of *Porichthys*. Basement membrane, b.m.; mucous cell, mu.c.; pigment cell, pg.c.; luminous cells, lum. or.; lens cells, l.c.; reflector, ref. After Greene.

large, and the highly refractive mass of lens cells behaves like a real lens. There is a reflected spot of intense light from each photophore when the fish is examined in sunlight. The lens could be excised and mounted in normal saline, where Greene observed that "it condenses sunlight to a bright point a distance back of the lens of from one-fourth to one-half its diameter." Histology of the luminous organs of *P. porosissimus* has been studied by Chiarini and Gatti (1899).

Embryology. Greene (1899) has followed the development of the light organs of *P. notatus* in detail. He found that the photophores arise quite late in development of the embryos. The first stages of the ventral rows appear in embryos 8.5 to 8.9 mm long after the sensory Anlage of the lateral line system have appeared. They arise "by local proliferation of cells from the epidermis in the region which they will permanently occupy." This cell group multiplies rapidly, forming a nodule which projects from the surface as a papilla, with subsequent

separation from the epidermis. The papilla elongates and lens cells differentiate from the gland cells proper. Figure 183 is a series of stages in development of the organ.

Physiology. Greene (1899) made many attempts to excite the luminescence of *Porichthys* kept in aquaria at the Hopkins Marine Laboratory, Pacific Grove, California, but in only one case, a fish pressed against the side of the tank, did a faint glow appear. However, if interrupted induced shocks are applied to a fish on a glass plate, the line of organs on ventral and lateral surfaces all glow beautifully with a white light. The single well-developed organ just back of and below the eye is especially prominent. No light is visible in organs that appear rudimentary in structure. The shocks must be very strong to excite luminescence, as an intensity sufficient to cause violent contractions of muscles has no effect on the photophores. Galvanic stimulation on make and break of the current is also effective.

The light does not appear suddenly but only after eight to ten seconds. It then gradually increases in intensity and gradually dims when the stimulation ceases. This behavior led Greene to attribute excitation by the current to a direct effect on the organ rather than an indirect one through possible nerves which could not be demonstrated histologically. The light can also be excited by addition of ammonia to the sea water. After a considerable latent period, the glow of all organs appears, remains at a maximum for a few minutes, and then for twenty minutes gradually fades. Rubbing the skin will again call forth luminescence.

The above behavior is typical of fish caught among the rocks where they guard the young brood. Two specimens from deep water in Monterey Bay could not be made to phosphoresce either with electrical or ammonia treatment, suggesting a seasonal activity of the photophores. However later experiments of Greene and Green (1924) have indicated that there is no seasonal activity of photophores, which are active in February, March, and April as well as in June and July, provided they are stimulated in the proper way.

Apparently the normal control of *Porichthys* photophores is by means of hormones. In coming to this conclusion the earlier experiments on electrical stimulation were repeated and the slow development of light confirmed. Greene and Greene state "The length of the latent period and the general character of the wave of development and waning of phosphorescence are suggestive of a chemically controlled gland rather than of one under nerve control. In order to test this hypothesis 0.25 cc. adrenalin hydrochloride was injected subcuta-

neously into the belly wall of the right side immediately under the prominent gastric line of phosphorescent organs. After a rather pronounced latent period, two or more spots above the area of injection began to show luminescence. These organs were not contiguous but well separated in the row. A few seconds later . . . individual organs in adjacent lines began to glow faintly, then several more organs in the posterior segment of the gastric line under which the injection occurred became visible. From this time on individual organs on both sides of the abdomen, of the anal lines, and mandibular lines, that is, in regions distant from the point of injection but still in irregular areas, became visible. It was noteworthy that no true sequence or order or uniformity in the degree of light intensity occurred at first. In the course of approximately ten minutes, the entire phosphorescent organ line system became visible and of uniform brightness." The illumination produced by adrenalin injection persisted for hours without interruption and in one experiment it was found that pituitrin also excited the luminescence.

These experiments have opened the way for an extended study of photophore control, not only in fish but in shrimp and squid, i.e., wherever chromatophores are present and make up a system whose hormone control is well known. Unfortunately little additional work has been carried out, chiefly because of lack of material. The author (1931) has tested a few deep sea fish, one of which responded to adrenaline by luminescence. No chemical experiments have been undertaken to determine the nature of the photogenic material in *Porichthys*.

Isospondyli and Iniomi. These orders are divided into several suborders and a large number of families, containing most of the deep sea fish with definite photophores. The best-known "lantern fishes" belong to the suborders, Stomiatoidea and the Myctophoidea, but a few of the Clupeoidea and Salmonoidea, related to the Stomiatoidea, contain luminous species, and possibly suborders related to the Mycetophoidea may contain genera with luminous species. These genera are indicated by italics in the general classification of teleosts. Little is known regarding the luminous organs and the emission of light has not been observed. It is possible that certain apparently mucous cavities may contain luminous material, and a study of living fish is much to be desired.

Both Gunther (1888) and Brauer (1906) divided the deep sea fish with numerous photophores into three principal families, the Stomiidae, Sternoptychidae, and the Scopelidae. Since then these groups have been divided into many families, but for consideration of their

luminescence will be treated together. The history of the discovery of deep sea fish and the controversy over the function of the pearly spots has already been given. It is sufficient to point out again that every part of the body may contain a photophore, even the most unexpected regions, the tongue or edge of the eye. As in the squid, an organ near the eye is almost always present and seems to be one of the most important and functionally active of all the photophores. Like the squid also the most usual position for photophores is ventral and lateral, but among fish there is a greater tendency to form lines of light, like the portholes on a ship.

Photophores are usually imbedded in the skin, but those of *Photostylus pycnopterus* of the Alepocephalidae are elevated on stalks (Beebe, 1933). In addition to body photophores, some of the stomiatoid fishes possess a barbel projecting from the lower jaw, with a light organ on the end. The barbel of *Macrostomias calosoma* (Beebe, 1933) is many branched at the tip and must when glowing resemble a tassel of luminous threads. Some of the many luminous species are shown in Fig. 184.

In addition to lights of barbels and the larger prominent photophores, there may be innumerable minute photophores scattered among the larger ones. Finally, Beebe (1933, 34) has frequently referred to luminous tissue on scales, such as those of *Bathylagus glacialis* of the Argentinidae, to luminous mucus and particularly to luminescence at the base of the teeth, presumably due to mucous material, in such fish as *Echiostoma*, *Melanostomias*, and *Idiacanthus*.

Classification. The Stomiatoidea may be divided into 10 families. In the following list luminous genera are italicized.

Gonostomatidae (*Bonapartia*, *Zaphotias*, *Cyclothone*, *Gonostoma*, *Photichthys*, *Yarella*, *Margrethia*, *Manducus*, *Triplophos*)

Sternoptychiidae (*Argyropelecus*, *Sternoptyx*, *Polyipnus*)

Astronesthidae (*Astronesthes*, *Neonesthes*, *Borostomias*, *Radonesthes*, *Diplolychnus*)

Chauliodontidae (*Chauliodus*)

Stomiidae (*Macrostomias*, *Bathylechnus*, *Stomias*, *Stomiatoides*, *Dactylostomias*, *Pachystomias*, *Haplostomias*, *Opostomias*)

Malacosteidae (*Aristostomias*, *Malacosteus*, *Photostomias*, *Ultimostomias*)

Idiacanthidae (*Idiacanthus*)

Melanostomiidae (*Bathophilus*, *Chirostomias*, *Echiostoma*, *Eustomias*, *Flagellotomias*, *Lamprotorus*, *Leptostomias*, *Melanostomias*, *Aristostomias*, *Photoneustes*, *Tactostoma*, *Odontostomias*, *Trigonolampa*, *Grammatostomias*, *Thysanactis*, *Pterostomias*)

Maurolicidae (*Ichthyococcus*, *Maurolicus*, *Valenciennellus*, *Vinciguerra*, *Argyrimus*, *Lychnopoles*, *Diplophos*)

Opisthoproctidae (*Opisthoproctis*)

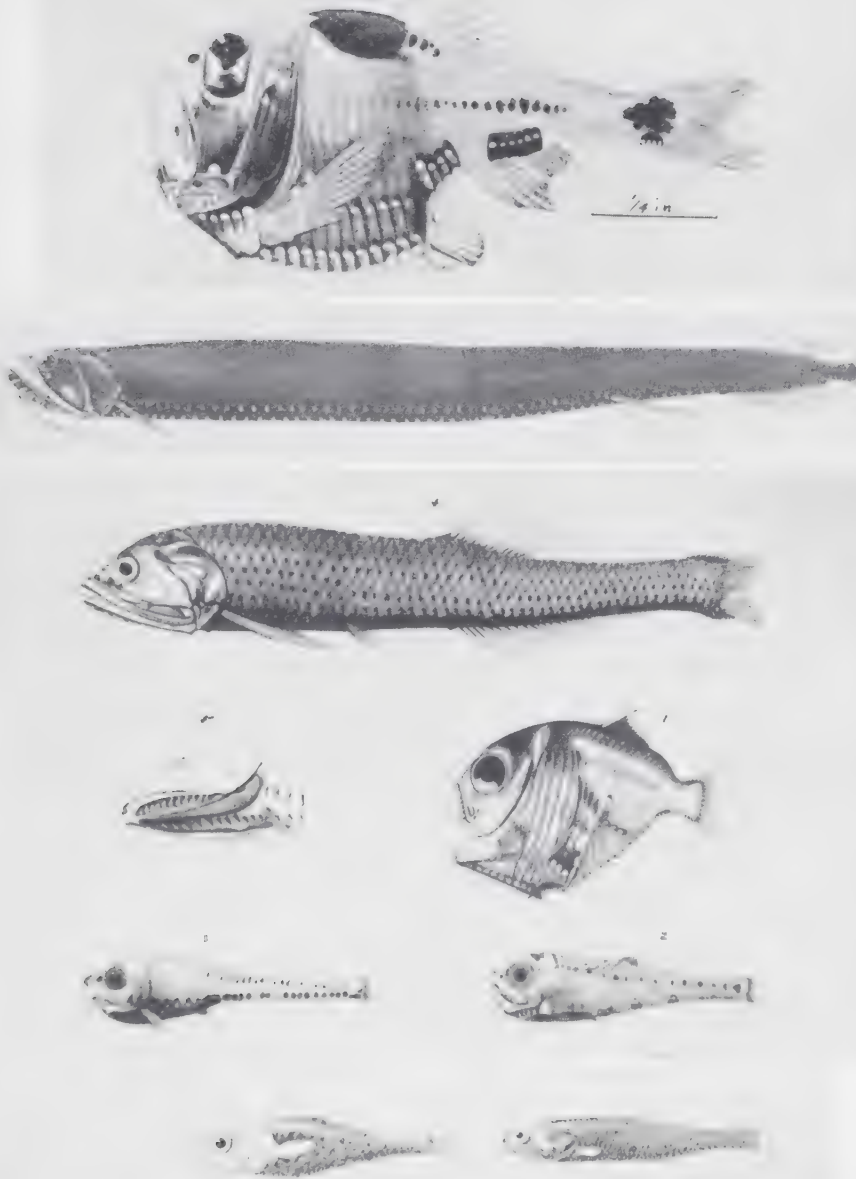


FIG. 184. Various types of deep sea fish with photophores. Top, *Argvrolepecus heathii*, next, *Stomias columbrinus*; 4, *Lychnopoles argenteus*; 1, *Sternoptyx obscura*; 3, *Maurolicus oculatus*; 2, *Valenciennellus stellatus*; bottom, right, *Myctophum laternatum*, and left, *M. oculatum*. From various sources.

The suborder Mycetophyoidea is divided into 4 families with luminous genera italicized, as follows:

Aulopidae (*Aulopus*)

Synodontidae (*Synodus*)

?*Sudidae* or *Paralepidae* (*?Ipnops*, *Sudis*, *Ludiosudis*, *Macroparalepis*, *Paralepis*, *Bathysauropsis*, *Chlorophthalmus*, *Notosudis*, *Benthosaurus*, *Bathypterois*, *Bathymicrops*, *Lestidium*)

Myctophidae or *Scopelidae* (*Diaphus*, *Lampadena*, *Lampanyctus*, *Scopelopsis*, *Scopelogadus*, *Myctophum* with subgenera *Diogenichthus*, *Electrona*, *Notoscopelus*, *Neoscopelus*, *Dasy Scopelus*, *Centrobranchus*, *Tarletonbeania*, etc.)

Histology. The systematic investigators of the fine structure of photophores are von Lendenfeld (1887), Brauer (1908), Emery (1884, 68, 90), Chiarini and Gatti (1899), Gatti (1899, 1904), Brandes (1899), Chiarini (1900), Handrick (1901), Mangold (1907), Trojan (1915), Ohshima (1911), Boulenger (1913), and Nussbaum-Hilarowicz (1920, 23). Mangold (1910), Trojan (1906, 29), Rauther (1927), and Penners (1931) have reviewed the subject fully.

The genera whose light organs have been studied in greatest detail are collected in the following table. The capital letters indicate the histologist who has studied them, according to the following key: L, von Lendenfeld; B, Brauer; Bo, Boulenger; E, Emery; H, Handrick; M, Mangold; O, Ohshima; T, Trojan; N, Nussbaum-Hilarowicz.

Stomiatoidea

Gonostomatidae [*Cyclothone acclinidens* (L, B), *C. signata* (T, N), *Gonostoma elongatum* and *denudatum* (B), *Photichthys argenteus* (B), *Triplophos elongatum* (B)]

Maurolicidae [*Lychnopoles argenteolus* (L), *Diplophos taenia* (B), *Ichthyoceros ovatus* (B), *Vinciguerrria lucetia* (B), *Valenciennelus* sp. (B), *Maurolicus pennantii* (M, O)]

Sternoptychidae [*Argyropelecus hemigymus* (L, B, H, N), *Sternoptyx diaphana* (L, B, N), *Polyipnus spinosus* (B)]

Stomiidae [*Opisthomias micripnus* (L), *Pachystomias microdon* (L), *Stomias leuconotus* (L), *S. valdiviae* (B), *Bathylchnus cyaneus* (B), *Dactylostomias ater* (B), *Macrostomias longibarbus* (B), *Lamprotopus flagellibarba* (Bo)]

Chauliodontidae [*Chauliodus barbatus* (L, B)]

Astronesthidae [*Astronesthes niger* (L), *A. lucens* (B)]

Melanostomiidae [*Echiostoma barbatus* (L), *Melanostomias melanops* (B)]

Idiacanthidae [*Idiacanthus antrostomus* (L), *I. fasciola* (B)]

Malacosteidae [*Malacosteus indicus* (L, B)]

Mycetophyoidea

Myctophidae [*Myctophum* (*Scopelus*) *benoiti* (L, B, E), *M. Watasei* (O)]

A pioneer in the field of histology was von Lendenfeld (1887), who recognized a number of types of photophores, depending on structure and position. These might be simple in structure, with or without a

pigment covering, or compound organs with or without a reflector. Some types were definitely glandular, on the body or lower jaw, on barbels or fin rays, or suborbital in positions, with or without a reflector. The same fish might possess organs of many different types. Von Lendenfeld considered the organs to be glands converted for light production and to have been developed (adapted) from the mucus canal system.

Brauer, whose work was reviewed in a popular article by Zugmayer (1910), also used the terms simple ("einfach") and compound ("zusammengesetzte") photophores. The simple organs, found among the Stomiidae (in Brauer's sense), are a mass of transformed epithelial cells, without a lens or reflector and usually without a pigment mantle, but some of the larger simple organs like that in the tip of the barbel of *Stomias* and *Idiacanthus* do have a pigment mantle. The movable postorbital organ of *Astronesthes* is partially surrounded by pigment and capable of rotation by a muscle, so as to cut off the light. The relatively large suborbital or postorbital organs of *Chauliodus*, *Idiacanthus*, *Dactylostomias*, *Malacosteus*, and the opercular organ of *Bathylchnus* were classed as simple by Brauer although there is a certain amount of complication. Truly simple organs are found scattered over the whole body and on the fins of *Chauliodus*, *Idiacanthus*, and the anterior body regions of *Lamprotopus*.

The compound organs, found in all three of Brauer's families, are made up of accessory structures, such as lens, reflectors, and pigment screens. They might be shaped like a cup, a flask, a paper bag, or a shell.

Another distinction of Brauer had to do with the presence or absence of an opening duct, the open or closed type of light organ. Classification would be simple if photophores were either open or closed, but there are actually all gradations between masses of cells without a lumen and without an opening, to those with a lumen and a long open duct. Intermediate conditions are found in which the duct is closed at the end or closed throughout its length or is (in *Argyropelecus*) a mere strand of cells, a remnant of what was formerly a duct. Despite a typical open gland structure, reflectors and lenses are also present. The open and closed type of photophore are shown in Fig. 185.

A great many different types of organs may be found in one species. *Gonostoma* for example has small and large cup-shaped organs with an open duct as well as a bag like organ at the base of the tail fin, a folded epithelial sac without a duct. There is also in *Gonostoma* a combination of the two types, with the sac like organ connected to the duct of the cup like organ.

Among scopelids, the organs may have a duct, like that on the



FIG. 185. Section of light organs of fish. Above, *Stomias*, without a duct. Below, *Cyclothone*, with a long duct turning to right. After Brauer. dr₁, dr₂, luminous gland cells; l, lens cells; p, pigment sheath.

margin of the tongue of *Neoscopelus* or may be ductless as in the ventral region of *Myctophum*. The large organ near the tail differs in position in the sexes and may be considered a secondary sexual character. Emery thought that scopelid photophores were mesodermal in origin as are electric organs, but the work of Gatti, Brauer, and others leaves little doubt but that all are derived from ectoderm.

Light organs are always well supplied with blood vessels, but the innervation, according to Brauer, is rather sparse and represented by branches of skin nerves. An exception may be *Myctophum* in which Ohshima has described the photogenic body as richly supplied by nerves and "undoubtedly controlled by nerves." Ray (1950) also, in a careful study of the nervous system of the myctophid, *Lampanyctus leucopsarus*, found all the photophores to be innervated, those of the head by branches of the facial and those of the body by spinal nerves. Even though the light organs of the body form a linear series, they are innervated by the ventral root of the spinal nerve of the body segment in which they are located.

The most recent detailed work on anatomy and histology of deep sea fish including photogenic organs, has been carried out by Nussbaum-Hilarowicz. Beautiful colored plates of stained sections showing the fine structures of the cells in the light organs of *Argyrolepecus hemigymnus*, *Sternoptyx diaphana* and *Cyclothone signata* were published in 1920, and similar sections of the barbel of *Stomias boa* in 1923.

Embryology. Roule and Angel (1930) have described many larval forms, and Beebe (1934) obtained stalk-eyed larvae of *Idiacanthus* and young of many different species, but the most extensive and systematic work on development of luminous organs in deep sea fish is due to Sanzo (1912 to 1935). His long and painstaking studies in Messina have supplied stages of development from eggs, through larvae of 10 mm length and adolescents to adult sizes of many centimeters. In addition to a number of Scopelids, (*Myctophum*, *Saurus*, *Chlorophthalmus*, *Aulopus*) the larval forms of *Bathophilus*, *Stomias*, *Gonostoma*, *Vinciguerria*, *Chauliodus*, *Ichthyococcus*, *Argyrolepecus*, and *Mauroliscus* have been described in detail. In general, luminous organs appear in quite young larval stages, for example, in *Gonostoma denudatum*, whose adult length may be 170 mm, between the 13- and 16-mm stage. A thoracic organ near the ventral fin and an opercular organ are the first to appear, followed by additional thoracic and caudal organs and a mandibular organ. For details the reader must refer to the original papers.

Physiology. Very little is known of the physiology of deep sea fish with photophores and what has been observed varies considerably, pos-

sibly depending on the condition of the animals. An early study was made by Mangold (1907) at Naples, using *Maurolicus pennantii*. There was no spontaneous luminescence nor did light appear on lifting the fish out of the sea water onto a glass plate, but strong electrical or mechanical stimulation gave a local response of those photophores near the point of stimulation, followed by others at a distance. Mangold was perplexed by this behavior, as histological study shows only sparse innervation of photophores, merely branches of skin nerves which do not look like true efferent photogenic nerves. He found that light was not excited by concentrated salt solution or 1% pilocarpine, cocaine, or atropine in sea water or dilute H_2SO_4 , but immersion in fresh water brought forth a long-lasting luminescence. The light disappeared completely on death, and it was not possible to work with an excised organ, like a nerve muscle preparation.

Ohshima (1911) made a few experiments with freshly caught *Maurolicus pennantii* on a fishing boat. Like Mangold, he noted no spontaneous luminescence, but a weak yellowish green light appeared on stimulation. Contrary to Mangold's finding, there was no light in fresh water until a few drops of formalin had been added, when a continuous feeble glow came from the photophores.

Among the *Myctophidae* also (particularly *Myctophum wataseni*), there was no spontaneous light, but on squeezing the fish or removing the brain, the anteorbital organs and luminous scales emitted a weak blue light while the brachiotegals, operculars, and other disc-like organs become very weakly luminescent. The mediocaudal photophores (Leuchtplatten of Brauer) were imperfectly developed. Ohshima wrote: "By the mode of action one can distinguish two groups of the organs, the anteorbitals and the luminous scales (and probably also the mediocaudals) on the one hand, and all the other small organs on the other. The two groups act quite independently of each other, while within each all the photophores become simultaneously active. A weak stimulation was followed by the emission of light, only from the second group, while the first group could be forced to action only by a stronger stimulation." There was no luminescence when the fish was placed in fresh water or dilute formalin.

Skowron (1928) has studied *Chauliodus sloanii* at Messina. These fish lived about forty-five minutes in a container of sea water. They swam briskly at first, later sinking to the bottom of the vessel. In a dark room a continuous blue light could be seen along the undersurface of the fish. This light was not increased on local stimulation with a glass rod, but other light organs at the point of excitation became visible and a wave of blue light spread from this point to both the com-

pound and the simple photophores. After some seconds the photophore lights went out, leaving only the weak continuous light on the belly. The stimulation could be repeated with a second lighting up of the photophores. Neither sunlight nor light from an arc lamp had any inhibiting effect on the rapidity or intensity of the luminescence.

The continuous blue light on the ventral side of the body probably comes from the cup-like organs, but some bottle-like organs may also be involved, as Skowron found that the latter remained luminescent for a long time after stimulation. The continuous light on the belly disappeared when the fish sank to the bottom of the vessel and could not be revived by strong stimulation at a time when other photophores responded. When the fish died, no photophores would light on strongest mechanical stimulation or by placing the fish in fresh water or by treatment with ether, chloroform, or ammonia. Adding a few drops of ammonia to sea water always caused the fresh fish to light for some ten seconds.

The most extended observations on method of lighting of fish, particularly myctophids, come from Beebe's studies during the cruise of the *Arcturus* in 1925 (Beebe and Pyl, 1944) and in Bermuda (Beebe and Crane, 1939). It was soon apparent that individual lights or groups of lights could be flashed on independently of others and that the light appeared to serve different purposes. *Myctophum coccoi* was particularly abundant and viable, and Beebe has given the following account of luminescence in this species:

"Scattered over the body are many small round luminous organs, which we may divide into three general sets. First, 32 ventral lights on each side of the body, extending from the tip of the lower jaw to the base of the tail; second, about 12 lateral lights arranged irregularly along the head and body, and third, a series of three to six median light plates or scales, either above or below the base of the tail.

"The lower battery, when going full blast, casts a solid sheet of light downward, so strong that the individual organs could not be detected. Five separate times when I got fish in a large, darkened aquarium, I saw good-sized copepods and other organisms come close, within range of the ventral light, then turn and swim still closer to the fish, whereupon the myctophid twisted around and seized several of the small beings.

" . . . Perhaps the best distinction between various species of lanternfish is the arrangement of the lateral light organs, and in the dark-room in absolute darkness, I could tell at a glance what and how many of each species were represented in a new catch, solely from their luminous hieroglyphics. When several fish were swimming about, these

side port-holes were almost always alight, and it seems reasonable that they may serve as recognition signs, enabling members of a school to keep together, and to show stray individuals the way to safety.

"The light scales of the tail are apparently of considerable importance. Ordinarily when the whole fish is glowing with the pale greenish light of luminescence, these caudal light are seldom seen. A clue to their use is found in the fact that they show a sexual difference, the males having them on the upper side of the peduncle and the females on the lower side. . . .

"In the dark it was thus possible to distinguish species of lantern-fish by the lateral hieroglyphics and the sexes by the upward or downward direction of the tail lights. I have never seen the latter illumination given out by fish swimming alone in an aquarium. Although it is very evident that the caudal flashes have some sexual significance, yet another very important function seems that of obliteration. . . . When the ventral lights die out they do so gradually, so that the eye holds the image of the fish for a time after their disappearance, but the eye is so blinded by the sudden flare of the tail lights that when they are as instantly quenched, there follow several seconds when our retina can make no use of the faint, diffused, remaining light, but becomes quite blinded. A better method of defense and escape would be difficult to imagine."

That deep sea fish do react to dim lights is indicated by Beebe's observations on *Myctophum* affine, which lived ten minutes in an aquarium. "For a considerable time it showed no light and then the entire fish was outlined by almost every one of the 80-odd organs. Then it would become completely dark and light up again, this occurring about every 15 seconds. Rarely several spark-like rays shone forth, penetrating, clear and brilliant. I happened to lift my wrist watch with its dully luminous dial close to the fish and it reacted at once, giving out two strong discharges of the caudal glands. Concealing the watch and later displaying the light of its face resulted in instant reaction. This happened eight times. I then flashed on my much stronger flash-light with no result. For five minutes I alternated the two artificial sources of illumination with identical results, the fish reacting vigorously to the watch dial, but paying no attention to the electric torch."

Most luminous deep sea fish are rather small (2-3 in.) but species of the genus *Echiostoma* may be a foot in length. Beebe (1926) has caught a number of *Echiostoma tanneri* from the *Arcturus* and observed in the dark the "warm reddish glow" of its wedge-shaped rose-colored suborbital or cheek organ. The author (1931) has had an

opportunity of studying two living individuals of *Echiostoma ctenobarba*, entirely black in color, caught at 800 fathoms south of Bermuda, and brought to the Beebe laboratory on Nonesuch Island in iced sea water. In addition to the prominent cheek organ, *Echiostoma* has two rows of large photophores along the ventral and lateral walls beside numerous minute photophores scattered over practically the whole body including the dorsal surface. The cheek organ, pink in life, was observed to flash rhythmically with a decidedly bluish luminescence when the fish was handled, especially when lifted out of the sea water. No other luminescence of any kind could be noted, however, despite the fact that the fish was squeezed and twisted to stimulate it strongly. A hypodermic needle was then inserted, but no luminescence additional to that of the cheek organ appeared. However, when 5 minims adrenaline (1:1000 in physiological salt) was injected with the hypodermic into the side, about one-third toward the tail end, there immediately appeared a yellowish luminescence of photophores locally, near the point of injection, and soon practically all the photophores of the fish were luminescing with a yellowish moderately intense continuous glow. This lasted a few minutes and then went out and could not be excited again by rubbing or handling, but appeared as before on a second, third, and fourth injection of adrenaline. The last injection was of 10 minims and excited all organs and also the pectoral and ventral fins. There is no doubt of the luminescence of these fins, despite the fact that they do not possess any marked visible organs. No luminescence was observed in the tail, anal fins, long pectoral rays, or barbel or lower jaw. The cheek organ flashed at intervals after adrenaline injection but did not change in rhythm or in any noticeable way. The flashing of this organ is not due to unscreening of a continuously luminous surface. The light appears and disappears on the organ itself, and for this reason we may presume that *Echiostoma* is self-luminous and does not harbor luminous bacteria as is the case in the Dutch East Indian fish, *Photoblepharon* and *Anomalops*.

A most striking action of adrenaline on *Argyrolepecus olfersi* has recently been described by Bertelsen and Grøntved (1949). No luminescence was visible until after injection, when nearly all the photophores emitted a greenish yellow luminescence, brightest from the abdominal and lateral series. A photograph (reproduced as Fig. 186) of the light was obtained, which showed the bright beam due to the lens and a fan-shaped portion, which came from the silvery reflector of the photophore. An artist's conception of the light is shown in Fig. 187.

It should be mentioned that adrenaline is not a stimulant for light

production after a fish has been dead some time. Skowron (1925) observed no light from recently caught but dead *Chauliodus*, and the author tested other species, even a feebly moving *Linophryne*, with negative results.

Biochemistry. There have been no biochemical studies on deep sea fish. Except in the case of *Malacocephalus* and the *Anomalopidae* containing luminous bacteria, the luciferin-luciferase reaction has not been tested in any luminous fish, deep sea or surface form. The only positive information has to do with the presence of fluorescent material in the photophores of *Myctophum* (*Scopelus*) *benoiti*. The author (1926) examined freshly caught specimens of this fish, which could

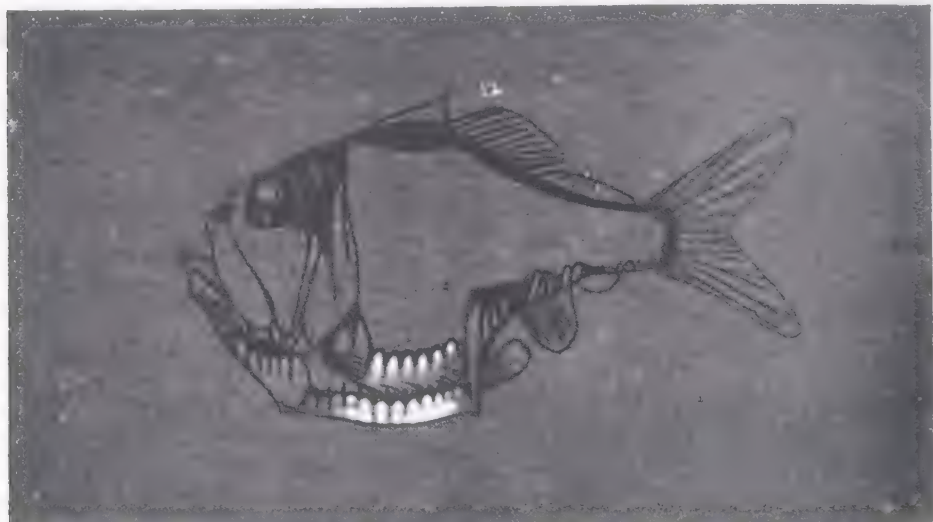


FIG. 186. *Argyropelecus olfersi*, photographed by its own light, with an outline of the fish drawn in. Photo by E. Bertelsen and J. Grøntved.

not be made to luminesce even by tearing them to pieces, and noted in ultraviolet light without the visible a strikingly bright greenish fluorescence of the lateral and ventral photophores. The photogenic cells and not the epidermal lens were the source of the fluorescence as could be determined by dissection. The use of ultraviolet light to pick out possible luminous organs is most convenient but not absolutely reliable. An *Argyropelecus hemigymnus* in the same moribund condition as *Myctophum* showed no fluorescence of any photophore.

The fluorescence in fish photophores is marked even in specimens preserved in formalin, at least this is true of *Myctophum benoiti*, *Stomias boa*, *Chauliodus sloani*, *Coccia ovata*, *Maurolicus pennanti*, *Vinciguerrria attenuata*, *V. poveriae*, and *Gonostoma denudatum*.

Another general fact whose significance is uncertain is the color of the photophores. Originally they were described as "pearly organs"

but there is great variation in color. Beebe (1934) has described the fresh serial and branchiostegal photophores of *Idiacanthus fasciola* as "blue violet, those of the ventral series being set in gilt frames," while the upper and lower caudal lights were "bright golden yellow." The colors purple to violet seem to be characteristic of serial photophores of the *Idiacanthidae*, *Astronesthidae*, and *Malacosteidae* (Beebe and Crane, 1939), the one exception observed being the genus *Bathophilus* where the color was golden yellow. The cheek organ of *Echi-*



FIG. 187. The hatchet fish, *Argyropelecus hemigymnus* as it would appear with its photophores illuminated. After Dahlgren, from a drawing by Bruce Horsfall.

ostoma is usually described as pink or red, perhaps a result of its rich blood supply. Although color may vary, it is interesting to find yellow appearing so frequently, in view of the rather wide distribution of yellow pigments in luminous organs of many invertebrates and the yellow color of highly purified Cypridina luciferin preparations. A wealth of information and a table of the color of luminous organs of fish with notes on luminescence will be found in the monograph of Beebe and Crane (1939).

Use of the Light. While the luminescence of floating plankton forms—Noctiluca, jelly fish, siphonophores, even *Philirrhoe*—present

real problems as regards the use of light to the animals, the free-swimming deep sea shrimp, cephalopods, and fish, with their elaborate photophore system, must use the light for a very definite purpose. That the barbel and tentacle organs are lures for feeding or to attract males seem very likely.

The most reasonable use of photophores among the stomiatids and scopolids is for recognition, a purpose strongly supported by Brauer (1904). Every species of deep sea fish has a characteristic distribution of photophores. The pattern of lights must take the place of the pattern in color of surface fish. This color pattern is largely due to chromatophores which in fish are under both nervous and hormone control, with changes of color pattern particularly noticeable during excitement and stress. Among deep sea forms, also, nervous control of the photophores seems to be secondary to hormone control, as indicated by the remarkable response to adrenaline among the few fish which have been tested. Observations on living fish in good condition indicate that there can be a selection of the lights or groups of lights which are turned on. Moreover, just as surface fish with color patterns show sexual dimorphism, so deep sea forms also have sexual differences in lighting pattern.

If the photophores are connected with recognition, the eyes of deep sea fish must be functional. Brauer's (1908) study of the eyes indicated that most luminous forms do have fully functional eyes and that many are oversize. Occasionally a deep sea fish will have eyes of the "telescopic" type, like those of *Argyrops*, and young fish may have their eyes on long stalks. These long stalk-eyed fish were named *Stylophthalmus* and referred by Brauer (1906) to the *Stylophthalmidae* until Beebe (1933) obtained evidence to indicate that they were young of *Idiacanthus*, and the short stalk-eyed fish were young of *Bathylagus* of the *Argentinidae*. This stalk-eyed condition is also found among young shrimp, crabs, squid, and gastropods.

It is the bathybenthonic forms which show the greatest reduction in size of eyes, with a few like *Bathymicrops regis* and *Ipnotis murrayi* actually blind. Fish with light organs also decrease in abundance at great depths. The whole subject of the relation of eye size to depth and to light production is a complicated one which cannot be considered here. The reader will find an excellent discussion in Murray and Hjort (1912).

Another use for certain of the photophores of fishes has been suggested by Beebe—a defense mechanism. The sudden momentary glare of tail lights in front of a predator might blind the attacker for a

moment and thus allow the luminous form to escape by a sudden turn in the darkness after its light has been quenched.

In special cases a light may have a special purpose, such as the photophore on the tongue of *Neoscopelus*, which may lure small animals directly into the mouth, a suggestion of Brauer (1908, p. 153). As in the case of squid, fish also have light organs on the edge of the eye—orbital organs—and in positions around the eye—preorbital, sub-orbital, and postorbital organs. They do not occur above the eye nor on the upper edge of the eye. Brauer (1904, 08) has devoted considerable space to a discussion of the use of orbital organs. They are present in all fish with body photophores except the *Myctophidae* (and even these fish have preorbital organs) so that they must have some relation to the body photophores. It is an interesting fact that in *Cyclothone obscura*, where the body photophores are retrogressive, the orbital photophores are also degenerate.

Brauer (1904) has discounted the suggestion of Pütter that orbital photophores throw light into the eye, thus aiding in detection of weak lights like a tapetum, pointing out that such a process would actually hinder vision. He did offer a somewhat similar hypothesis, that if the trunk organs emitted a colored light and orbital organs did also, it might enable the eye to recognize the colored lights of the same species more easily, and help distinguish the differently colored lights of another species, reminding the fish, so to speak, what color the lights of its relatives were. However, Brauer admitted that further observation would be necessary to establish this hypothesis.

The problem of the suborbitals or cheek organs is still a pressing one. As observations on *Echiostoma* show, the suborbital may function independently of the other photophores, which are excited by adrenaline. The author had supposed that perhaps light organs near the eye were indicators to tell when other photophores were lit, but the behavior of *Echiostoma* does not bear out this hypothesis. It must be admitted that the bionomic significance of these special photophores is still unknown.

ADDENDA

Since the manuscript of Bioluminescence went to press, details of the ultraviolet absorption spectrum of Cypridina luciferin have been published by Chase and Brigham (1951). McElroy, Coulombre and Hays (1951) have found 10 times as much pyrophosphatase (changing pyrophosphate to orthophosphatate) in the lantern of the fire-fly than in the rest of the body, and Schneyer (1951) has studied the effect of low concentrations of calcium and phosphate on bacterial luminescence intensity. These references are in the bibliography, together with a reference to a paper by Schertel (1902) on luminous fungi in literature and myth.

Papers in press include a demonstration by chromatography of two types of luciferin by H. S. Mason (Biol. Bull., Woods Hole), a study of the luminescence intensity of fungi, bacteria and Cypridina luciferin and luciferase as a function of oxygen pressure by J. W. Hastings (J. Cell. Comp. Physiol.) and a study of oxygen consumption of luminous bacteria using the oxygen electrode, by M. J. Goodkind and E. N. Harvey (J. Cell. Comp. Physiol.). In addition, W. D. McElroy has examined in more detail the part played by phosphate groups in fire-fly luminescence (J. Biol. Chem.).

BIBLIOGRAPHY

The attempt has been made to include in the following list all the important papers on light production by animals and plants from 1800 to and including 1947. A few short notices concerning the occurrence of luminous organisms, chiefly fire-flies, in a certain locality have been omitted and only a few of the systematic monographs on groups containing luminous species are included. It is a difficult task to find all publications in a field, and the author hopes that only few papers on histology, physiology, and biochemistry of luminous tissues have been overlooked. Short bibliographies will be found in Ehrenberg (1834), Dittrich (1880), Gadeau de Kerville (181, 87) on insects, Mangold (1910), Harvey (1920, 24, 40), Pratje (1923), Dubois (1928), Johnson (1947) on bacteria, and Buck (1948) on fire-flies.

Bibliography*

- Acloque, A. 1905. Le ver luisant. *Cosmos*, Paris, N.S., 53: 677-679.
- Acloque, A. 1907. La Lumiere des Insects. *Cosmos*, Paris, N.S., 56: 624-626.
- Agassiz, A. 1874. Embryology of the Ctenophorae. *Mem. Amer. Acad. Arts & Sci.* 10: Pt. 2 Supp. 357-398.
- Agassiz, L. 1850. On the structure of the Halcyonoid polypi. *Proc. Amer. Ass. Adv. Sci.* 3: 207-213.
- Akabane, T. 1938. A supplement note on the nature of light produced by luminous bacteria obtained from the deep-sea fish of the family, Macrouridae. *Jap. J. Physiol. (Tokyo)*: 3, 310-317. In Japanese.
- Alcock, A. 1899. A descriptive catalogue of the Indian deep sea fishes in the Indian Museum. Calcutta. Also in *Ann. Mag. Nat. Hist.* (Ser. 6) 4: 376-99, 450-61.
- Alcock, A. 1902. A naturalist in Indian seas. London. 238 pp.
- Alcock, A. 1892-1908. Illustrations of the zoology of the "Investigator" under command of Carpenter and Hoskyn. Fishes, Part I to IX. Government Printing Office, Calcutta.
- Alenitzyn, W. D. 1875. Über das Leuchten von Diptera. *Dtsch. Ent., Z.* 19: 432.
- Alexander, G. 1935. Is a pacemaker involved in synchronous flashing of fireflies? *Science*, 82: 440.
- Alexander, R. S. 1943. Factors controlling fire fly luminescence. *J. cell. comp. Physiol.*, 22: 51-70.
- Allard, H. A. 1920. The flight of fire flies and the flashing impulse. *Science* 52: 539-540. Also 44: 710, 1916.
- Allard, H. A. 1931. The photoperiodism of the fire fly *Photinus pyralis* Linn; its relation to the evening twilight and other conditions. *Proc. ent. Soc. Wash.* 33: 49-58.
- Allard, H. A. 1935. Synchronous flashing of fire-flies. *Science* 82: 517-518.
- Allman, G. J. 1844. [luminous earthworms.] *Rep. Brit. Ass. Cork*, 1843, 76.
- Allman, G. J. 1851. On the emission of light by *Anurophorus*. (*Leptura*) *fimentarius*, Nicolet. *Proc. R. Irish Acad.* 5: 125.
- Allman, G. J. 1862. Note on the phosphorescence of *Beroë*. *Proc. roy. Soc. Edinb.* 4: 518-519.
- Allman, G. J. 1871. A monograph of the gymnoblastic or tubularian hydroids. Part I. Phosphorescence, p. 145-6. Ray Society, London.
- Allman, G. J. 1872. Notes on Noctiluca. *Quart. J. Micr. Sci. (N.S.)* 12: 326-332.
- Amberson, W. R. 1922. Kinetics of the bioluminescent reaction in *Cypridina*. I. and II. *J. gen. Physiol.*, 4: 517-558.
- Anderson, R. S. 1933. The chemistry of bioluminescence. I. Quantitative determination of luciferin. *J. cell. comp. Physiol.*, 3: 45-59.
- Anderson, R. S. 1935. Studies on bioluminescence. II. The partial purification of *Cypridina* luciferin. *J. gen. Physiol.*, 19: 301-305.

* For abbreviations of journals the World List of Scientific Periodicals has been used.

- Anderson, R. S., and E. N. Harvey. 1934. The effect of deuterium oxide on the luminescence of luciferin. *J. cell. comp. Physiol.* 5: 249-253.
- Anderson, R. S. 1936. Chemical studies on bioluminescence. III. The reversible reaction of Cypridina luciferin with oxidizing agents and its relations to the luminescent reaction. *J. cell. comp. Physiol.*, 8: 261-276.
- Anderson, R. S. 1937. Chemical studies on bioluminescence. IV. Salt effects on the total light emitted by a chemiluminescent reaction. *J. Amer. Chem. Soc.* 59: 2115-2117.
- Anderson, R. S. 1948. Chemiluminescence in aqueous solutions. *Ann. N.Y. Acad. Sci.* 49: 337-352.
- Anderson, R. S., and A. M. Chase. 1944. The nature of Cypridina luciferin. *J. Amer. Chem. Soc.* 66: 2129.
- Annandale, Nelson. 1900. Observations on the habits and natural surroundings of insects made on the "Skeat Expedition" to the Malay Peninsula, 1899-1900. VI. Insect luminosity (an aquatic lampyrid larva). *Proc. zool. Soc. Lond.* 1900. 862-865.
- Annandale, N. 1906. Notes on the freshwater fauna of India. III. An Indian aquatic cockroach and beetle larva. *J. Asiat. Soc. Beng. (N.S.)* 2: 106-107.
- Anonymous. 1832. On the luminous appearance of the roots of the garden lettuce. *Graves' Naturalist's J.* 1: 18-20.
- Anonymous. 1903. Investigations of luminous bacteria. *Sci. Amer. Supp.* 55: 22777.
- Anon. (Paul Popenoe). 1917. The fireflies' light. *J. Hered.* 8: 368-372.
- Apstein, C. 1909. Die Pyrocysteen der Plankton Expedition. *Ergebn. Plank.-Exp. Humboldt-Stiftung.* 4Mc. 1-27.
- Araki, T. 1950. The effect of light on the luminous solution of Cypridina hilgendorfi. *Annot. zool. jap.* 23: 98-103.
- Arambourg, C. 1920. Sur un Scopelide fossile a organes lumineux: *Myctophum ternatum* n.sp. du Sahelien oranais. *Bull. Soc. Geol. Fr. (Ser. 4)* 20: 167-168. 233-239.
- Arambourg, Camille. 1929. *Argyropelecus logearti*, un nouveau poisson bathypelagique du Sahelien. *Bull. Soc. Geol. Fr. (4)* 29: 11-15.
- Archangeli, G. 1889. Ricerche sulla fosforescenza del *Pleurotus olearius* D. C. *Mem. Accad. Lincei* 286: 611.
- Arndt, W. 1924. Leuchtende Tausendfüsse in Schlesien. *Jh. Ver. Schles. Insektenk.* 14: 31-33.
- Arnold, C. 1881. Beiträge zur vergleichende Physiologie. *Mitt. Naturf. Ges. Bern.* No. 979-1003, pp. 151-192. Sec. 3 Das Verhältnisse des Sauerstoffs zur phosphoreszenz, pp. 174-178.
- Asbjørnsen, P. C. 1856. *Brisinga endecacnemos*. *Fauna litoralis Norvegiae*, Vol. 2, Bergen.
- Atkinson, G. F. 1887. Observations on the female form of *Phengodes laticollis*. *Horn. Amer. Nat.* 21: 853-856.
- Atkinson, G. F. 1887. A remarkable case of phosphorescence in an earthworm. *J. Elisha Mitchell Sci. Soc.* 4: 89-91. Also in *Amer. Nat.* 21: 773-774.
- Atkinson, G. F. 1889. Another phosphorescent mushroom. *Bot. Gaz.* 14: 19.
- Audouin, M. V. 1840. Remarques sur la phosphorescence de quelques animaux articulés, a la occasion d'une lettre de Forester sur la phosphorescence des Lombrics terrestres. *C. R. Acad. Sci. Paris* 11: 747-749.

- Aue. 1918. Weitere Beobachtung über die Leuchtfähigkeit von *Arctia caja* L. Ent. Z. 32: 69-70.
- Aue. 1922. Besitzt der Falter von *Arctia caja* die Fähigkeit zu leuchten? Biol. Zbl. 42: 141-142.
- Azara, F. de. 1809. De voyages dans l'Amerique Méridional par Don Felix de Azara, depuis 1781 jus qu'en 1801. Paris. Vol. I, 114-221.
- Baart de la Faille, J. M. 1821. De Animalibus phosphorescentibus. Groningae, 79 pp.
- Baba, K. 1935. Report of the Biological survey of Mutsu Bay. 27 Nudibranchia. Sci. Rep. Tohoku Univ. Ser. 4: Biol. 10: 331-336.
- Bačkovsky, J. M., J. Komárek, and K. Wenig. 1939. Attempt to explain the mechanism of the luminescence in Vejdovsky's earthworm. Vestn. Čsl. Zool. Spolecn. 7: 10 pp.
- Bade, E. 1918. Deep-sea fish with lanterns. Pop. Sci. Mon. 92: 358-359.
- Bage, F. 1904. Notes on phosphorescence in plants and animals. Vict. Nat. 21: 93-104.
- Bahl, K. N. 1943. Pheretina, an Indian earthworm. (Indian Zool. Mem.) 3rd ed., 17-18.
- Baird, W. 1830-1831. On the luminousness of the sea. Mag. Nat. Hist., London. 3: 308-321 and 4: 500-511, 1831.
- Baird, W. 1843. Note on the luminous appearance of the sea, with descriptions of some of the entomostracous insects by which it is occasioned. Zoologist 1: 55-61.
- Baird, W. 1848. Note on the genus *Cypridina* M. Edwards with a description of two new species. Ann. Mag. Nat. Hist. (Ser. 2) 1: 21-25.
- Balduf, W. V. 1935. Bionomics of *Entomophagus* Coleoptera. pp. 87-105.
- Ball, E. G., and P. A. Ramsdell. 1944. The flavin-adenine dinucleotide content of firefly lanterns. J. Amer. Chem. Soc., 66: 1419.
- Ballner, F. 1907. Über das Verhalten von Leuchtbakterien bei der Einwirkung von Agglutinationsserum und anaesthetisierenden chemischen Agentien nebst Bemerkungen über Pflanzennarkose. Zbl. Bakt. (2nd Abt.) 19: 572-576.
- Balss, H. 1944. Decapoda Leuchtorgane. Bronn's Klassen u. Ordnung des Tierreichs, Leipzig, 5 (Abt. 1, Buch 7): 661-667.
- Bancel, C., and C. Husson. 1879. Sur la phosphorescence de la viande de homard. C. R. Acad. Sci., Paris 88: 191-192.
- Barber, H. S. 1906. Note on *Phengodes* in the vicinity of Washington, D.C. Proc. Ent. Soc. Wash. 7: 196-197.
- Barber, H. S. 1908. The Glow-Worm *Astraptor*. Proc. Ent. Soc. Wash. 9: 41-43.
- Barber, H. S. 1913. A new species of *Phengodes* from California (Coleoptera). Canad. Ent. 45: 343-345.
- Barber, H. S. 1913. Luminous collembola. Proc. Ent. Soc. Wash. 15: 46-50.
- Barber, H. S. 1923. A remarkable wingless glow-worm from Ecuador. (Coleoptera, Lampyridae.) Insec. Inscit. Menst., 11: 191-194.
- Barnard, H. K. 1925. A monograph of the marine fishes of South Africa. Ann. S. Afr. Mus. 21: 1-1065.
- Barnard, J. E. 1899. Photogenic Bacteria. Trans. Jenner Inst. Lond., 25: (Ser. 2): 81-112.
- Barnard, J. E. 1902. Luminous bacteria. Nature, Lond. 65: 536-538; also in Knowledge, 34: 190-192, 1911.
- Barnard, J. E., and A. Macfadyen. 1902. On luminous bacteria. Ann. Bot. Lond. 16: 587-589; also in Rep. Brit. Ass. Adv. Sci. (1903), 801.

- Barrois, Th. 1890-1891. Sur le presence de *Lumbricus* (*Photodrilus*) phosphorescent à Groffiers (Pas de Calais) Rev. Biol. Nord France, 3: 117-119.
- Bates, H. W., and others. 1864. No title (on Fulgura). Trans. R. Ent. Soc. Lond., 2: XIII-XIV proc.
- Baylor, E. R. 1949. The growth cycle of luminous bacteria on limited substrate. Thesis. Princeton Univ.
- Beach. 1874. The Cocuyo. Sci. Rec. 3: 485.
- Becker, J. J. M. 1848. [Note on Fulgura] Ann. Soc. Ent. Fr. (Ser. 2), 6: Bull. p. XIV.
- Becquerel, E. 1867. La Lumière, ses causes et ses effects. Vol. 1 Sources; vol. 2, (1868) Effets. Paris.
- Beddard, F. E. 1899. A note upon phosphorescent earthworms. Nature, Lond. 60: 52.
- Beebe, W. 1926. The Arcturus Adventure, pp. 215-219. New York.
- Beebe, W. 1929. Deep-sea fish of the Hudson Gorge. Zoologica, N.Y., 12: 19 pp. (No. 1).
- Beebe, W. 1932. The depths of the sea. Nat. Geogr. Mag., 61: 65-89, see also 62: 741-758 (1932).
- Beebe, W. 1932. Nineteen new species and four post-larval deep-sea fish. Zoologica, N.Y. 13: 47-107.
- Beebe, W. 1933. Deep-sea isospondylous fishes. Zoologica, N.Y. 13: 159-167.
- Beebe, W. 1933. Deep-sea fishes of the Bermuda oceanographic expeditions. I. Introduction. Zoologica, N.Y. 16: 5-11.
- Beebe, W. 1933. Deep-sea fishes of the Bermuda oceanographic expeditions. II. Family Alepocephalidae. Zoologica, N.Y. 16: 15-93.
- Beebe, W. 1933. Deep-sea fishes of the Bermuda oceanographic expeditions. III. Family Argentinidae. Zoologica, N.Y. 16: 99-147.
- Beebe, W. 1933. New data on the deep sea fish, *Stylophthalmus* and *Idiacanthus*. Science 78: 390.
- Beebe, W. 1934. Three new deep-sea fish seen from the bathysphere. Bull. N.Y. Zool. Soc. 37: 190-193.
- Beebe, W. 1934. Half Mile Down. New York. See also: Nat. Geogr. Mag., 66: 661-704 (1934).
- Beebe, W. 1934. Deep-sea fishes of the Bermuda oceanographic expeditions. IV. Family Idiacanthidae. Zoologica, N.Y. 16: 149-241.
- Beebe, W. 1937. Preliminary list of Bermuda deep-sea fish. Based on the collections from fifteen hundred metre-net hauls, made in an eight-mile circle south of Nonsuch Island, Bermuda. Zoologica, N.Y. 22: 197-208.
- Beebe, W., and J. Crane. 1939. Deep-sea fishes of the Bermuda oceanographic expeditions. Family Melanostomiidae. Zoologica, N.Y. 24: 65-238.
- Beebe, W., and J. Crane. 1947. Eastern Pacific expeditions of the New York Zoological Society XXXVII. Deep-sea Ceratioid fishes. Zoologica, N.Y. 31: 151-184.
- Beebe, W., and M. Vander Pyl. 1944. XXXIII Pacific Myctophidae. Zoologica, N.Y. 29: 59-95.
- Behning, A. 1929. Über eine leuchtende Chironomide des Tschalkar-Sees. Zeit. Wiss. InsektBiol. 24: 62-65.
- Benjerinck, M. W. 1889. Les bacteries lumineuses dans leur rapports avec l'oxygene. Arch. Néerl. Sci. 23: 416-427.

- Beijerinck, M. W. 1889. Le photobacterium luminosum, bacterie lumineuse de la mer du nord. Arch. Néerl. Sci. 23: 401-415.
- Beijerinck, M. W. 1889. L'auxanographie ou la méthode de l'hydrodiffusion dans la gelatine appliquée aux recherches microbiologique. Arch. Néerl. Sci., 23: 367-372.
- Beijerinck, M. W. 1890. Over lichtvoedseeln plastisch voedsel van Lichtbacterien. Proc. Acad. Sci. Amst. Afdeel. Natuurk. 2de Reeks, Deel 7: 239, 1890.
- Beijerinck, M. W. 1891. Sur l'aliment photogène et l'aliment plastique des bactéries lumineuses. Arch. Néerl. Sci., 24: 369-442.
- Beijerinck, M. W. 1902. Photobacteria as a reactive in the investigation of the Chlorophyll-function. Proc. Acad. Sci. Amst. 4: 45-49.
- Beijerinck, M. W. 1912. Mutation bei Microben. Folia Microbiol. 1: 1-97, esp. 49-54.
- Beijerinck, W. M. 1915. Die Leuchtbakterien der Nordsee im August und September. Folio Microbiol. 4: 15-40.
- Beijerinck, M. W. 1917. The enzyme theory of heredity. Proc. Acad. Sci. Amst. 19: 1275-1289.
- Bell, F. J. 1881. The eye-like spots in fishes. Pop. Sci. Rev., 20 (N.S. 5): 221-234.
- Bell-Marley, H. W. 1913. Some notes on a luminous South African fulgorid insect (*Rinortha guttata*, Walk) together with a description of its parasitic lepidopterous larva. (Ser 4) 17: Zoologist, 281-309.
- Benham, W. B. 1899. Phosphorescent earthworms. Nature, Lond. 60: 591.
- Bennett, F. D. 1833. On the light emitted by a species of *Pyrosoma*. Proc. Zool. Soc. Lond. (Part 1): 79-80.
- Bennett, F. D. 1837. On marine *Noctilucae*. Proc. Zool. Soc. Lond. 5: 1-3, 51-52. Also in Phil. Mag. (Ser 3) 12: 212-213, 1838.
- Bennett, F. D. 1840. Narrative of a whaling voyage around the world from the year 1833 to 1836. London. 2 vols. pp. 401 and 395.
- Berkeley, M. J. 1857. Introduction to cryptogamic botany, p. 265. London.
- Berlese, A. 1909. Gli Insetti. Vol. I. Embriologia e morfologia. Milano. pp. 709-711, Organi luminosi.
- Bernoulli, C. 1803. Über das Leuchten des Meeres u.s.w. Göttingen. 6 pts.
- Berry, S. S. 1913. Nematolampas, a remarkable new cephalopod from the South Pacific. Biol. Bull. Wood's Hole 25: 208-212.
- Berry, S. S. 1920. Light production in Cephalopods, I and II. Biol. Bull. Wood's Hole 38: 141-195.
- Berry, S. S. 1926. A note on the occurrence and habits of a luminous squid (*Abralio veranyi*) at Madeira. Biol. Bull. Wood's Hole 51: 257-268.
- Bertelsen, E. 1943. Notes on the deep-sea angler-fish *Ceratias holbølli* Kr. based on specimens in the Zoological Museum of Copenhagen. Vidensk. Medd. Naturh. Foren. Kbh. 107: 185-206.
- Bertelsen, E., and Jul. Grøntved. 1949. The light organs of a bathypelagic fish *Argyropelecus olfersi* (Cuvier) photographed by its own light. Vidensk. Medd. Naturh. Foren. Kbh., 111: 163-167.
- Berthelot, A., and G. Amoureux. 1924. Influence de sodium pyruvate sur le bacterie phosphorescente. Bull. Soc. Chim. Biol. Paris 6: 336-7.
- Bethune, C. J. S. 1863. Luminous larvae. Canad. Ent. 1: 38-39.
- Bigg-Wither, T. P. 1878. Pioneering in South Brazil. London.

- Bischof, G. 1823. Ueber die Phosphoreszenz der unterirdischen Rhizomorphen. Schweiggers Neu. J. Chem. Phys., 39 (N.S. 9): 259-305.
- Bischoff, G. W., Nees v. Esenbeck, Noggerath. 1823. Die unterirdischen Rhizomorphen, ein leuchtender Lebensprozess. Nova Acta Leop. Carol. 11: (Part 2), 603-712 and 12: 2, 875. Abstract in Flora, Jena 2: 419, 1924. See also Flora, Jena 1: 115-123, 1923.
- Blackman, V. H. 1902. Observations on the Pyrocystae. New Phytol. 1: 178-188.
- Blair, K. G. 1915. Luminous insects. Nature, Lond. 96: 411-415. Also in Proc. S. Lond. Ent. Nat. Hist. Soc. 1914-15: 31-45.
- Blair, K. G. 1924. Some notes on luminosity in insects. Ent. Mon. Mag. 60: 173-178.
- Blair, K. G. 1926. On the luminosity of Pyrophorus (Coleoptera). Ent. Mon. Mag. 62: 11-15.
- Blair, K. G. 1927. An aquatic glowworm. Nat. Hist. Mag. 1: 59. Also in Trans. R. Ent. Soc. Lond. 75: 43-45.
- Blakeslee, A. L. 1948. Stars of Death. Exploring the breathtaking lovely grottoes of Waitomo Cave in New Zealand—the home of the glow-worm. Nat. Hist. N.Y. 57: 75-77.
- Boeck, A. 1865. Oversigt over de ved Norges kyster iagttage Copepoder henhørende til Calanidernes Cyclopidermes og Harpactidernes familier. Forh. Vidensk. Selsk. Krist. 7: 226-282.
- Böckmann, C. W. 1800. Beobachtung und Versuche über das Leuchten des faulenden Holzes in gasarten und tropfbaren Flüssigkeiten. Scherer's Alleg. J. Chem. 5: 3-35. Also Phil. Mag. 16: 18-26, 146-154 (1803).
- Boisduval, J. A. 1833. De la phosphorescence de certaines chenilles de Mamestra oleracea, L. Gen. Rev. Ent. 1: 226. Also Ann. Soc. Ent. Fr. 1832: 424.
- Bolin, R. L. 1939. A review of the myctophid fishes of the Pacific Coast of the United States and of Lower California. Stanford Ichthyol. Bull. 1: 89-160.
- Bongardt, J. 1903. Beiträge zur Kenntnis der Leuchtorgane einheimischer Lampyriden. Zeit. wiss. Zool. 75: 1-45.
- Bongardt, J. 1904. Zur Biologie unserer Leuchtkäfer. Naturw. Wschr. 19: 305-310.
- Bonhomme, C. 1940. Nouvelles recherches biologiques et cytologiques sur la luminescence de quelques Polynoinae. Bull. Acad. Montpellier Dec. 9. No. 20: 77-83.
- Bonhomme, C. 1942. Researches sur l'histologie de l'appareil lumineux des Polynoinés. Bull. Inst. Oceanogr. Monaco 39: No. 823, 8 pp.
- Bonhomme, C. 1943. L'appareil lumineux de Chaetopterus variopedatus Clap. Recherches histologiques. Bull. Inst. Oceanogr. Monaco No. 843, 1-7.
- Bonhomme, C. 1944. La luminescence de *Heterocirrus bioculatus* Keferstein. Bull. Inst. Oceanogr. Monaco 41: No. 871, 7 pp.
- Born, E. 1911. Beiträge zur feineren Anatomie der Phyllirhoë bucephala. Zeit. wiss. Zool. 97: 105-197. Also in S.B. Ges. Naturf. Fr. Berl. 1907.
- Borodin, N. 1931. Atlantic Deep Sea Fishes. Bull. Mus. Comp. Zool. Harv. 72: 53-89.
- Bory de St. Vincent, J. B. 1804. Voyages dans les quatres principales Isles des mers Afrique. Paris, I, p. 112.
- Bosc, L. A. G. 1800. Histoire des vers. Suite de Buffon.
- Bose, S. R. 1926. Luminous leaves and stalks from Bengal. Nature, Lond. 117: 156-157.

- Bose, S. R. 1930. Relation of sunlight to the light of luminous wood. *Naturwissenschaften* 18: 787.
- Bose, S. R. 1935. Photograph of a luminous fungus by its own light. *Sci. Cult.* 1: 57.
- Bose, S. R. 1935. A luminous agaric (*Pleurotus* sp.) from South Burma. *Trans. Brit. Mycol. Soc.* 19: 97-101.
- Bothe, F. 1928. Über den Einfluss des Substrats und einiger anderer Faktoren auf Leuchten und Wachstum von *Mycelium X* und *Agaricus melleus*. *S.B. Akad. Wiss. Wien Abt. I.*, 137: 595-626.
- Bothe, F. 1930. Eine neuer einheimischer Leuchtpilz, *Mycena tintinnabulum*. *Ber. dtsh. bot. Ges.* 48: 394-399.
- Bothe, F. 1930. Der leuchtende Olbaumpilz, *Clitocybe olearia* DC auf künstlichen Nährböden. *Z. Pilz.* 14: (N.S. 9): 81-84.
- Bothe, F. 1931. Über das Leuchten verwesender Blätter und seine Erreger. *Planta* 14: 752-765.
- Bothe, F. 1935. Genetische Untersuchungen über die Lichtentwirkung der Hutpilze I. *Arch. Protistenk.* 85: 369-383.
- Boué, A. 1869. Über das gefärbte See-wasser und dessen Phosphoreszenz im Allge mein. *S.B. Akad. der Wiss. Wien Abt II* 49: 251-262.
- Boulenger, C. L. 1913. The luminous organs of *Lamprotoxus flagellibarba*. *Fish. Ireland Sci. Invest.* 1912, no. 2. 2 pp.
- Bowden, B. J. 1950. Some observations on a luminescent freshwater limpet from New Zealand. *Biol. Bull. Wood's Hole* 99: 373-380.
- Bowles, G. H. 1882. On luminous insects. *Rep. Ent. Soc. Ont.*, 34-37.
- Bowring, J. C. 1844. Note on *Fulgora candelaria*. *Ann. Mag. Nat. Hist. (Ser. 1)* 14: 425.
- Boyer, J. 1897. La lumiere du ver luisant et les rayons X. *Nature, Paris* 25: 180-181.
- Boyer, J. 1934. Les pyrophores. *Lanternes vivantes des forêts tropicales. Nature, Paris* 62: 31-32.
- Brade-Birks, H. K. and S. G. 1916-18. Notes on Myriapoda VI and XII. *Lancs. Chesh. Nat.* 10: 113; 11: 152-165, 186-192.
- Brade-Birks, H. K. and S. G. 1920. Luminous Chilopoda. *Ann. Mag. Nat. Hist. (Ser 9)* 5: 1-30.
- Brandes, G. 1899. Die Leuchtorgane der Tiefseefische *Argyrolepecus* und *Chauliodus*. *Naturw.* 71: 447-452. Also in *Verh. d. Zool. Ges.* 9: 247-248 (1899).
- Brandt, J. F. 1838. Ausführliche Beschreibung der von C. H. Mertens auf seiner Weltumsegelung Schirmquallen. *Mem. Acad. Sci. St.-Pétersb. (Ser. 6)* 4: (Sc. Nat.) 2nd part, 237-411. Leuchten der Schirmquallen, 306-315.
- Brandt, K. 1885. Die Koloniebildenden Radiolarien (Sphaerozoen) des Golfes von Neapel. *Fauna u. Flora Neapel*, 13: 1-276. Phosphoreszenz, pp. 136-139.
- Branner, J. C. 1885. The reputation of the lantern-fly. *Amer. Nat.* 19: 834-838.
- Branner, J. C. 1910. The luminosity of termites. *Science* 31: 24-25; 32: 342.
- Brauer, A. 1902. Diagnosen von neuen Tiefseefischen, welche von der Valdivia-Expedition gesammelt sind. *Zool. Anz.* 25: 277-298.
- Brauer, A. 1904. Ueber die Leuchtorgane der Knochenfische. *Ver. Dsch. Zool. Ges.* 14: 16-35.
- Brauer, A. 1905. Die Gattung *Myctophum*. *Zool. Anz.* 28: 377-404.
- Brauer, A. 1906-1908. Die Tiefseefische. *Wiss. Ergebn. Valdivia. Jena*, 1908.

- I. Systematischer Teil. 15: Part 1 (1906). II. Anatomischer Teil., 15: Part 2 (1908).
- Brefeld, O. 1877. Botanische Untersuchungen über Schimmelpilze. Leipzig. 3: 136, 170.
- Bridge, T. W. 1922. Fishes. In *Camb. Nat. Hist.* 7: 139-420. pp. 178-181 on phosphorescent organs.
- Brightwell, Thomas FLS. 1857. On self-division in Noctilucae. *Quart. J. Microsc. Sci.* 5: 185-191.
- Britton, W. E. 1903. An interesting luminous insect. *Rep. Conn. Agric. Exp. Sta.* 268-271.
- Brockhausen, H. 1903. Über leuchtende Scolopender (a letter). *Jber. westf. Prov. Ver. Wiss. Kunst* 31: 163-164.
- Brooks, G. 1940. Observations sur la phosphorescence des trois derniers anneaux du ver luisant. *C. R. Acad. Sci. Paris* 210: 228-230.
- Brooks, W. K. 1893. The genus *Salpa*. A monograph. *Mem. Biol. Lab. Johns Hopk. Univ.* No. 2, 396 pp. and 57 pl.
- Brown, B. 1925. A luminous spider. *Science* 62: 182, 329; 63: 383.
- Brown, D. E. S., F. H. Johnson and D. A. Marsland. 1942. The pressure, temperature, relations of bacterial luminescence. *J. Cell. Comp. Physiol.* 20: 151-168.
- Brown, D. E. S., and C. V. King. 1931. The nature of the photogenic response of *Photuris pennsylvanica*. *Physiol. Zoöl.* 4: 287-293.
- Brown, F. J., Jr. 1950. Bioluminescence in *Comp. Animal Physiol.* Phila., pp. 660-676.
- Bruini, G. 1906. I batteri fosforescenti. *Riv. Igiene Sanit. Pubbl.* 17: 297-321.
- Bruner, L. 1891. Phosphorescent myriapods. *Insect Life* 3: 319-321.
- Bruner, L. 1908. Footnote. *Biol. Cent. Amer. Zool.* 52: Orthoptera 2: 286.
- Bryson, H. C. 1940. Bioluminescence. *Paint Manuf.* 10: 170-173.
- Buchet, S. 1937. Observations sur la luminosité et sur le pigment de "Clitocybe olearia." *Bull. mensuel Soc. Linn. Lyon*, 6: 44-46.
- Buchner, P. 1914. Sind die Leuchtorgane Pilzorgane? *Zool. Anz.* 45: 17-21.
- Buchner, P. 1921, 1930. Symbiosen bei Leuchtenden Tiersen. *Tier und Pflanze in Symbiose*. Berlin, 2nd ed. pp. 664-736; 1st ed. 1921, pp. 340-400.
- Buchner, P. 1922. Über das "tierische" Leuchten. *Naturwissenschaften* 10: 1-7, 30-34.
- Buchner, P. 1926. *Tierisches Leuchten und Symbiose*. Berlin, 58 pp.
- Buck, J. B. 1935. Synchronous flashing of fire-flies experimentally produced. *Science* 81: 339-340.
- Buck, J. B. 1937. Studies on the firefly. I. The effects of light and other agents on flashing in *Photinus pyralis* with special reference to periodicity and diurnal rhythm. *Physiol. Zoöl.* 10: 45-58.
- Buck, J. B. 1937. Studies on the firefly. II. The signal system and color vision in *Photinus pyralis*. *Physiol. Zoöl.* 10: 412-419.
- Buck, J. B. 1937. Flashing of fire-flies in Jamaica. *Nature, Lond.* 139: 801.
- Buck, J. B. 1938. Synchronous rhythmic flashing of fire-flies. *Quart. Rev. Biol.* 13: 301-314.
- Buck, J. B. 1941. Studies on the fire-fly. III. Spectrometric data in thirteen Jamaican species. *Proc. Rochester Acad. Sci.* 8: 14-21.
- Buck, J. B. 1946. The spiracular factor in the control of luminescence in the fire-fly. *Anat. Rec.* 96: 51.

- Buck, J. B. 1947. Studies on the fire fly. IV. Ten new lampyrids from Jamaica. *Proc. U.S. Nat. Mus.* 97: 59-79.
- Buck, J. B. 1948. The anatomy and physiology of the light organ in fireflies. *Ann. N.Y. Acad. Sci.* 49: 397-482.
- Buck, J. B. 1950. Control of luminescence in *Phengodes*. *Anat. Rec.* 108: 121-122.
- Buddenbrock, W. von. 1928. *Grundriss der vergleichenden Physiologie*. Berlin. 830 pp., pp. 459 to 469 *Lichtproduction*; 2nd ed. (1939), pp. 1110-1125.
- Bugnion, E. 1915. L'anatomie du Lampyre ou ver luisant. *Bull. Soc. vaud. Sci. Nat.* (5) 50: *Proc.-Verb.* 92-94.
- Bugnion, E. 1919. Le ver luisant provençal (*Phausis delarouzei* Duval). *C. R. Soc. Biol., Paris* 82: 994-999.
- Bugnion, E. 1920. Les organes lumineux du ver luisant provençal (*Phausis delarouzei* Duval). *Festschr. Zschokke*, #33, Basel, 19 pp.
- Bugnion, E. 1929. Le Ver-luisant provençal et la Luciole nicoise. *Riviera Sci. Mem.* I; suppl. 131 pp.
- Buhigas, R. S. 1918. Hematotalasia. *Mem. Soc. Esp. Hist. Nat.* 10: 407-458.
- Bujor, P. 1901. Sur l'organisation de la Vérétille. *Arch. Zool. Exp. Gen.* (Ser. 3) 9: Notes et revue No. 4, XLIX-LX.
- Bukatsch, F. 1936. Über den Einfluss von Salzen auf die Lichtentwicklung von Bakterien. *S.B. Akad. Wiss. Wien Abt. I*, 145: 259-276. Also, *Chemikerztg.* 61: 309 (1937).
- Buller, A. H. R. 1924. *Researches on Fungi*. London, Vol. III, The bioluminescence of *Panus stepticus*, Chap. 12, 357-431.
- Buller, A. H. R. 1934. *Researches on Fungi*. London, vol. VI. *Omphalia flava* a gemmiferous and luminous leaf-spot fungus, 397-454.
- Buller, A. H. R., and T. C. Vanterpool. 1926. The bioluminescence of *Omphalia flava*, a leaf spot fungus. *Phytopathology* 16: 63.
- Burbanck, W. D., and C. Lower. 1946. The American rail-road worm—a luminescent insect larva. *Bios* 17: 198-200.
- Burckhardt, C. R. 1900. On the luminous organs of Selachian fishes. *Ann. Mag. Nat. Hist.* (Ser. 7) 6: 558-568.
- Burge, W. E. 1916. Comparison of the intensity of oxidation in luminous and non-luminous insects. *J. Franklin Inst.* 182: 263-264.
- Burghause, F. 1914. Krieslauf und Herzsclag bei *Pyrosoma giganteum* nebst Bemerkungen zum Leuchtvermögen. *Zeit. wiss. Zool.* 108: 430-497.
- Burkenroad, M. D. 1936. The Aristaeinae, Solenocerinae and pelagic Penaeinae of the Bingham Oceanic Collection. *Bull. Bingham Oceanogr. Coll.* 5 (No. 2): 151 pp.
- Burkenroad, M. D. 1937. The Templeton Crocker Expedition XII Sergestidae (Crustacea decapoda) from the lower California region, with description of two new species and some remarks on the organs of Pesta in *Sergestes*. *Zoologica*, N.Y. 22: 315-329.
- Burkenroad, M. D. 1943. A possible function of bioluminescence. *Sears. Found. J. Mar. Res.* 5: 161-164.
- Burmeister, H. 1836. A manual of entomology. London. Trans. by W. E. Shuckard from *Handbuch der Entomologie*, 1832. The luminousness of insects, pp. 490-497.
- Burmeister, H. 1872. Observations on a light giving coleopterous larva. *J. Linn. Soc. (Zool.)* 11: 416-421. Also: *Stettin. Ent. Ztg.* 26: 344 (1875).

- Busch, W. 1847. Einiges Ueber Tomopteris onisciformis. Arch. Anat. Physiol. Lpz. 180-186.
- Busch, W. 1851. Beobachtung über Anatomie und Entwicklungsgeschichte einige wirbellosen Seethiere. Berlin, p. 103.
- Busk, G. 1852. Observations on certain points in the anatomy of a species of Thaumantias. J. R. Micr. Soc. 3: 22-25.
- Butler, E. A. 1895. The glowworm. Sci. Amer. Supplement 39: 15883-15884.
- Butschli, O. 1883-1887. In Bronn's Klassen und Ordnungen des Thier Reichs. Bd. I. Abt. ii; Mastigophora, 906-1029 (Dinoflagellata) Lichtproduction 1021-1022; 1030-1097 (Cystoflagellata).
- Butschli, O. 1885. Einige Bemerkungen über die gewissen Organisationsverhältnisse der Cilioflagellaten und der Noctiluca mit einem Beitrag von E. Askenasy. Morphol. Jahrb., 10: 529-573.
- Bütschli, O. 1921. Vorlesung über vergleichende Anatomie, I (lif 3) Leuchtorgane, 900-931, J. Springer, Berlin.
- Butt, C., and R. S. Alexander. 1942. A method of recording low intensity flashes of light. Rev. Sci. Inst., 13: 151-153.
- Calkins, G. N. 1899. Mitosis in Noctiluca miliaris and its bearing on the nuclear relations of the Protozoa and Metazoa. J. Morph., 15: 711-772.
- Calvert, P. P. 1925. Phengodes laticollis. Ent. News 36: 317.
- Cannon, H. G. 1931. On the anatomy of Gigantocypris mülleri. Discovery Rep. 19: 187-243.
- Cardot, H. and M. Lefèvre. 1929. Sur la fonction photogenique de certains peridiniens. Bull. Soc. Linn. Lyon 8: 48-50.
- Carradori, G. 1808. Esperienze ed osservazione sopra il fosforo delle Lucciole (*Lampyris*). Giorn. di Fis., Chim. e Storia Nat. Pavia 1: 269-282.
- Carradori, G. 1809. Dell'Azione di diversi fluidi gassosi sopra il fosforo delle Lucciole (*Lampyris italica*) e Luccioloni (*Lampyris splendidula*). Giorn. di Fis. Chim. e Storia Nat. Pavia 2: 247-264.
- Carradori, G. 1814. Dell'eccitabilita del fosforo della Lucciole. Giorn. di Fis. Chim. e Storia Nat. Pavia 7: 306-312.
- Carrara, M. 1836. Sulla phosphorezza della lucciole commune (*Lampyris italica* L.). Bibl. Ital. 82: 357-370.
- Carus, C. 1829. Ueber das Leucht der italienischen Leuchtkäfer. Carus Analect. Naturwiss. Heilk., 169-170.
- Carus, C. G. 1864. Expériences sur la matière phosphorescente de la *Lampyris italica*; action d'eau pour rendre a la matière dessechée cette phosphorescence. C. R. Acad. Sci. Paris 59: 607-608.
- Carus, J. V. 1868. Ueber Noctiluca miliaris Sur. Arch. mikr. Anat. 4: 351-352.
- Carus, P. 1901. Electricity and phosphorescence in the animal world. Open Court (Chicago, Ill.), 15: 540-550.
- Caullery, M. 1921. La symbiose chez les animaux. Bull. Inst. Past. 19: 569-583. 617-627.
- Causey, D. 1926. Mitochondria in Noctiluca scintillans (Macartney 1810). Univ. Calif. Publ. Zool. 28: 225-230.
- Chace, F. A., Jr. 1940. Plankton of the Bermuda Oceanographic Expeditions. IX. The Bathypelagic Caridean Crustacea. Zoologica, Stuttgart 25: 117-209.
- Chakravorty, P. N., and R. Ballentine. 1941. On the luminescent oxidation of luciferin. J. Amer. Chem. Soc. 63: 2030-2031.

- Champion, G. C. 1883. No title (on Fulgora). Trans. R. Ent. Soc. Lond., 1883. proc. xx.
- Chance, B., E. N. Harvey, F. H. Johnson, and G. Millikan. 1940. The kinetics of bioluminescent flashes. A study in consecutive reactions. J. Cell. Comp. Physiol. 15: 195-215.
- Chandler, B. 1908. Luminosity in plants. Trans. Bot. Soc. Edinb. 23: 333-338.
- Chase, A. M. 1940. Changes in the absorption spectrum of *Cypridina* luciferin solutions during oxidation. J. Cell. Comp. Physiol. 15: 159-171.
- Chase, A. M. 1941. Observations on luminescence in *Mnemiopsis*. Biol. Bull. Wood's Hole 81: 296.
- Chase, A. M. 1942. The reaction of *Cypridina* luciferin with azide. J. Cell. Comp. Physiol. 19: 173-181.
- Chase, A. M. 1943. The absorption spectrum of luciferin and oxidized luciferin. J. Biol. Chem. 150: 433-445.
- Chase, A. M. 1945. The visible absorption band of reduced luciferin. J. Biol. Chem. 159: 1-4.
- Chase, A. M. 1946. Reversible heat inactivation of *Cypridina* luciferase. J. Cell. Comp. Physiol. 27: 121-124.
- Chase, A. M. 1948. The chemistry of *Cypridina* Luciferin. Ann. N.Y. Acad. Sci. 49: 353-375.
- Chase, A. M. 1948. Effects of hydrogen ion concentration and of buffer systems on the luminescence of the *Cypridina* luciferin-luciferase reaction. J. Cell. Comp. Physiol. 31: 175-192.
- Chase, A. M. 1949. Studies on cell enzyme systems. I. The effect of ferricyanide on the reaction of *Cypridina* luciferin and luciferase and the combining weight of luciferin. J. Cell. Comp. Physiol. 33: 113-122.
- Chase, A. M. 1949. Studies on enzyme systems. II. Evidence for enzyme-substrate complex formation in the reaction of *Cypridina* luciferin and luciferase. Arch. Biochem. 23: 385-393.
- Chase, A. M. 1950. On the nature of *Cypridina* luciferin. Biol. Bull. Wood's Hole 99: 326-327. An abstract.
- Chase, A. M. 1951. Inactivation of *Cypridina* luciferase by alcohols. Fed. Proc. 10: 25.
- Chase, A. M., and E. H. Brigham. 1951. The ultraviolet and visible absorption spectra of *Cypridina* luciferin solutions. J. Biol. Chem. 190: 529-536.
- Chase, A. M., and A. C. Giese. 1940. Effects of ultraviolet radiation on *Cypridina* luciferin and luciferase. J. Cell. Comp. Physiol. 16: 323-340.
- Chase, A. M., and J. H. Gregg. 1949. Analysis of *Cypridina* luciferin for nitrogen. J. Cell. Comp. Physiol. 33: 67-72.
- Chase, A. M., and E. N. Harvey. 1942. A note on the kinetics of *Cypridina* luminescence. J. Cell. Comp. Physiol. 19: 1-2.
- Chase, A. M., and P. B. Lorenz. 1945. Kinetics of the luminescent and non-luminescent reactions of *Cypridina* luciferin at different temperatures. J. Cell. Comp. Physiol. 25: 53-63.
- Chase, A. M., J. Schryver, and K. G. Stern. 1948. Exploratory electrophoretic experiments on luciferase preparations. J. Cell. Comp. Physiol. 31: 25-34.
- Chaudoir. 1880. Monographie des Scaritides, Ann. Soc. ent. Belg. 23: 1-130.
- Chiaje, S. Delle. 1827. Memoria sulla storia e notomia degli animali senza vertebre del regno di Napoli. 3: 58.

- Chamm, P. 1900. Recherche sulla struttura degli organi fosforescenti dei pesci. Ric. Fisiol. Sci. Affini ded. a L. Lucian Milano, 381-402.
- Chiarini, P., and M. Gatti. 1899. Ricerche sugli organi biofotogenetici dei pesci. Parte 1: Organi di tipo ghiandolare. R. C. Accad. Lincei. S., 8: 551-556.
- Chierchia, G. 1885. Collezioni per studi di scienze naturali. Estratto dalla Riv. maritt. Sett. Ott. Nov., p. 107.
- Chodat, R., and A. de Coulon. 1916. La luminescence de deux bacteries. Arch. sci. phys. nat., 41: 237-239.
- Chun, C. 1880. Die Ctenophoren des Golfes von Neapel. Fauna u. Flora Neapel 1: 313 pp., 194-195.
- Chun, C. 1887. Die pelagische Tierwelt in grösseren Meerestiefen. Bibliogr. Zool. 1: 1-56. Also in: S. B. Akad. Wiss. Berlin 30 (1889).
- Chun, C. 1893. Leuchtorgan und Facettenauge. Ein Beitrag zur Theorie des Sehens in grossen Meerestiefen. Biol. Zbl. 13: 544-571.
- Chun, C. 1896. Atlantis. Biologische Studien über pelagische Organismen. Chap. VI. Die Leuchtorgane der Euphausiden. Zoologica, Stuttgart 7: 196-212.
- Chun, C. 1903. Aus den Tiefen des Weltmeeres. 2nd ed. Jena, pp. 565-574, Leuchtorganismen der Tiefsee.
- Chun, C. 1903. Ueber Leuchtorgane und Augen von Tiefsee-Cephalopoden. Verh. Dtsch. Zool. Ges. 13: 67-91.
- Chun, C. 1910. Die Cephalopoden. I Teil Oegopsida. Wiss. Ergeb. Valdivia Exped. 18: Text 1-402, Atlas, Leuchtorgane; 38-51.
- Chun, C. 1913. Cephalopoda. Rep. Sars N. Atlantic Deep Sea Exped. 3: 1-28.
- Cienkowski, L. 1871. Über Schwärmerbildung bei Noctiluca miliaris. Arch. mikr. Anat. 7: 131-139; 9: 47-61, 1873.
- Claparède, E., and J. Lachmann. 1858-1859. Etudes sur les Infusories et les Rhizopodes. Mem. Inst. Nat. Genev. 5 (mem. 3): 1-260; 6 (mem. 1): 261-482.
- Claren, O. B. 1938. Zum Stoffwechsel der Leuchtbakterien. I. Liebigs Ann. 535: 122-149.
- Clark, R. E. D. 1937. Phosphorescence of the sea. Nature, Lond. 139: 592.
- Claus, C. 1863. Über einige Schizopoden und niedere Malacostraken Messina's. Z. wiss. Zool. 13: 422-454.
- Claudian, M. 1896. Sur les bacteries lumineuses. Bull. Soc. Sci. Nat. Bruxelles, 1898: 11-15.
- Coblentz, W. W. 1909. Notiz über eine von der Feuerfliege herrührende fluoreszenzierende Substanz. Phys. Z. 10: 955-956, and Bull. U.S. Bur. Stand. 6: 321.
- Coblentz, W. W. 1909-1910. The light of the fire fly. Elect. World, N.Y. 54: 1184-1185; 56: 1012-1013.
- Coblentz, W. W. 1911. The colour of the light emitted by Lampyridae. Canad. Ent. 43: 355-360.
- Coblentz, W. W. 1912. A Physical Study of the Firefly. Publ. Carneg. Inst. No. 164: 3-47.
- Coblentz, W. W., and C. W. Hughes. 1926. Spectral energy distribution of the light emitted by plants and animals. Sci. Pap. U.S. Bur. Stand. 21: 521-534.
- Cocco, A. 1838. Su di alcuni Salmonidi del Mare di Messina, lettera al C. L. Bonaparte. Nuovi Ann. Soc. nat. Bologna 2: 161-194.
- Cohn, F. 1856. Die Welt im Wassertropfen. Triest, p. 43.
- Cohn, F. 1873. Leuchtende Regenwürmer. Z. wiss. Zool. 23: 450-461.
- Cohn, F. 1878. Verzameling van stukken betreffende het geneeskundig staatsrecht in Nederland, 1878, p. 126.

- Coldstream, Dr. 1847. Luminousness, Animal. R. B. Todd's Cycloped. Anat. Physiol. London, 3: 197-205.
- Collet, R. 1896. Poissons. Result Camp. Sci. Prince de Monaco, Fasc. 10, 198 pp.
- Collingwood, C. 1869. A luminous fungus from Borneo. J. Linn. Soc. (Bot.) 10: 469.
- Connell, C. H. and J. B. Cross. 1950. Mass mortality of fish associated with the protozoan *Gonyaulax* in the Gulf of Mexico. Science 112: 359-363.
- Conroy, J. 1882. The spectrum of the light emitted by the glowworm. Nature, Lond. 26: 319.
- Cook, O. F. 1900. Camphor secreted by an animal, *Polyzonium*. Science 12: 516-521.
- Cooper, D. and R. Cooper. 1838. On the luminosity of the human subject after death, with remarks and details of experiments made with a view to determining the nature of the fact. Phil. Mag. 12 (Ser. 3): 420-426.
- Corner, E. J. H. 1950. Descriptions of two luminous tropical Agarics. (*Mycena* and *Dictyopanus*) Mycologia 42: 423-431.
- Cotton, S. 1891. Contribution a l'etude des bacilles photogene. Bull. Pharm. Lyon 13: 76-79.
- Coulon, L. 1938. Sur quelques animaux curieux du musée (7 ser.). Le mole ou poisson-lune, *Orthogoriscus mola* L. Bull. Soc. Sci. Nat. Elbeuf. for 1937, 56: 95-96.
- Coupin, H. 1893. Un cephalopode lumineux. Nature, Paris 21: (2nd sem.): 99-100.
- Coupin, H. 1915. Projecteurs vivants. Nature, Paris 43 (2nd sem.): 135-138.
- Coutière, H. 1905. Note preliminaire sur les Encyphotes recueillis par S.A.S. le Prince de Monaco a l'aide du filet a grande ouverture. Bull. Mus. Oceanogr. Monaco, no. 48, 35 pp. and 1906, no. 70.
- Cowan, F. 1865. Curious facts in the history of insects including spiders and scorpions. Philadelphia.
- Crawshay, L. R. 1935. Possible bearing of a luminous syllid on the question of the landfall of Columbus. Nature, Lond. 136: 559-560.
- Creighton, W. S. 1926. The effect of adrenalin on the luminescence of fireflies. Science 63: 600-601.
- Crie, L. 1881. Sur quelque cas nouveaux de phosphorescence dans les végétaux. C. R. Acad. Sci., Paris 93: 853-854.
- Crie, L. 1882. La phosphorescence dans le règne végétal. Rev. Sci. Fr. Etr. 29 (Ser. 3) 3: 299-301.
- Cros, A. 1924. *Pelania mauritanica*—variations—moeurs—evolution. Bull. Soc. hist. Nat. Afr. N. 15: 10-52.
- Crozier, W. J. 1917. The photic sensitivity of *Balanoglossus*. Journ. exp. Zool. 24: 211-217.
- Crozier, W. J. 1920. Persisting rhythm of light production in balanoglossids. Anat. Rec. 20: 186.
- Cruickshank, J. 1934. A study of a luminous organism in relation to nutrition on agar. J. Path. Bact. 39: 141-148.
- Cunningham, J. T. 1911. Animal Life. Reptiles, Amphibians and Fishes. London. pp. 410-431.
- Curtis, J. 1827. An account of *Elater noctilucus*, the firefly of the West Indies. Zool. J. 3: 379-382.

- Czapek, F. 1909. Zur Kenntniss des Phytoplankton im Indischen Ozean. S.B. Akad. Wiss. Wien Abt. I. 118: 231-239.
- Czapek, F. 1925. Biochemie der Pflanzen. Jena, 3rd ed. 3: Lichtentwicklung. 53-57.
- Czepa, A. 1912. Organismenleuchten und Zweckmässigkeit. Naturw. Wschr. 27 (N.S. 11): 609-613.
- Dahl, F. 1893. Pleuromma, ein Krebs mit Leuchtorgan. Zool. Anz. 16: 104-109.
- Dahl, F. 1894. Leuchtende Copepoden. Zool. Anz. 17: 10-13.
- Dahlgren, U. 1908. The luminous organ of a new species of *Anomalops*. Science 27: 454-455.
- Dahlgren, U. 1915-1917. The production of light by animals. J. Franklin Inst. 180: 513-537; 717-727; 181: 109-125; 243-261; 377-400; 525-556; 659-699; 805-843; 183: 79-94; 211-220; 323-348; 593-624. 735-754.
- Dahlgren, U. 1922. Phosphorescent animals and plants. Nat. Hist. N.Y. 22: 5-26.
- Dahlgren, U. 1928. The bacterial light organ of *Ceratias*. Science 68: 65-66.
- Dahlgren, U., and W. A. Kempner. 1908. A text-book of the principles of animal histology. Tissues of light production, N.Y. pp. 122-140.
- Dale, J., J. Main, etc. 1834. The glow-worm. Mag. Nat. Hist., 250-253.
- Damm, O. 1914. The bacterial lamp, from Prometheus (Berlin). Amer. Rev. Revs., 49: 486-487.
- Dammermann, K. W. 1923. The fauna of Krakatau, Verlaten Island and Sebesy. Treubia 3: 61-112.
- Dana, J. D. 1846. On some genera of Cyclopacea. Ann. Mag. Nat. Hist. 18: 181-185.
- Dana, J. D. 1852. Crustacea of the U.S. Exploring Exped. 1838-1842. 13 (pt. 1). 598. 644; (pt. 2), 1295.
- Daniel, E. E. 1950. The reversal of norleucine inhibition in luminous bacteria. J. Cell. Comp. Physiol. 36: 41-58.
- Darste, C. 1855. Memoire sur les animacules et autres corps organisés qui donnent a la mer une couleur rouge. Ann. Sci. Nat. (Ser. 4) 3: 179-242.
- Davis, C. C. 1948. *Gymnodinium brevis* sp. nov., a cause of discolored water and animal mortality in the Gulf of Mexico. Bot. Gaz. 109: 358-360.
- Dawydoff, C. 1946. Contribution à la connaissance des Ctenophores pélagiques des eaux de l'Indochine. Bull. Biol. 80 (2): 114-170.
- Dean, B. Ed. by Gudger, E. W., and A. W. Henn. 1923. A bibliography of fishes. N.Y. 3 vols. Vol. III pp. 513-514 on Luminosity and phosphorescence.
- de Bellesme, J. 1871. Observation sur la phosphorescence des oeufs du Lampyre commun. C. R. Acad. Sci. Paris 73: 629-630.
- de Bellesme, J. 1880. Recherches expérimentales sur la phosphorescence du lampyre. J. Anat., Paris 16: 121-169; C. R. Acad. Sci., Paris 90: 318-321; Rev. Med. Fr. Etr. 1: 287-291.
- de Blainville, H. D. 1834. Manuel d'Actinologie, p. 82.
- de Blainville, H. D. 1838. Rapport sur les résultats scientifique du voyage de la Bonite autour du mond. C. R. Acad. Sci., Paris, 6: 445-477 on p. 458-460 Phosphorescence de la mer.
- de Coulon, A. 1916. Etude de la luminescence du *Pseudomonas luminiscens*. Thèse, Neuchatel, 95 pp.
- Deegener, P. 1925. Handbuch der Entomologie. 3 vols. Ed. C. Schröder, Berlin. Leuchtorgane, vol. I, pp. 424-429.

- Degner, E. 1923. Zur Entwicklung von *Histioteuthis*. Zool. Anz. 55: 215-220.
- De Ierna, B. 1937. Le recenti ricerche chimico fisiche sulla bioluminescenza (con d'analisi spettrale della luce emessa da *Bacillus sepiae* Zirp.) Riv. Fis. Mat. Sci. Nat. 11: 615-622.
- De Ierna, B. 1942. Il problema della fotogenesi batterica in zoologia generale. Boll. Zool. (Turin), 13: 75-80, 102-106.
- De Ierna, B. 1946. Ricerche spettrofotometriche sulla luce emessa da batteri fotogeni. Noto I and II. R.C. Accad. Lincei (Ser. 8) 1: 1343-1348; 2: 78-82 (1947).
- Della Valle, A. 1875. La Luce negli animali. Thesis, Napoli, 69 pp.
- Delsman, H. S. 1939. Plankton investigations in the Java Sea. Treubia 17: 139-181.
- Dennell, R. 1940. On the structure of the photophores of some decapod crustacea. Discovery Rep. 20: 307-382.
- Dennell, R. 1942. Luminescence in decapod crustacea. Sci. J. R. Col. Sci. 12: 60-68.
- Deslongchamps, J. A. 1838. Note concernant des pêches phosphorescentes. Mem. Soc. Linn. Normandie 6: 307-308.
- Dessaignes, J. P. 1809. Memoire sur le phosphorescences. V. De phosphorescences spontanees. J. de Physique 69: 25-35.
- Dewar, Sir James. 1910. Light reactions at low temperature. Not. Proc. Roy. Instn. 19: 921-928.
- Dimmock, George. 1889. Luminous eggs of Insects. Psyche, Lond. 5: 169-170.
- Dittrich, R. 1888. Über das Leuchten der Tiere. Wissenschaftliche Beilage zum Programm des Realgymnasiums am Zwinger zu Breslau. Breslau Progr. Nr. 200. 70 pp.
- Döderlein, L. 1885. Seeigel von Japan und den Liu-Kiu-Inseln. Arch. Naturgesch., 51: 73-112.
- Doenitz, W. 1868. Über *Noctiluca miliaris*. Sur. Arch. Anat. Physiol. Lpz. 10: 137-149.
- Doflein, F. 1906. Über Leuchtorgane bei Meerestieren. S.B. Ges. Morph. Physiol. München 3: 47-61.
- Doflein, F. 1900. Studien zur Naturgeschichte der Protozoen IV Zur Morphologie und Physiologie der Zell- und Kernteilung. Nach Untersuchungen an *Noctiluca* und anderen Organismen. Zool. J. Abt. 2, 14: 1-60.
- Doflein, F. 1906. Über Leuchtorgane bei Meerestieren. S.B. Ges. Morph. Physiol. München 22: 133-136.
- Doflein, F. 1914. Das Tier als Glied des Naturganzen. In Tierbau und Tierleben, of R. Heffe and F. Doflein, vol. II. Leuchtorgane und Leuchtende Tiere, 889-893.
- Dohrn, C. A. 1868. *Fulgora Mitrii* Burm. Stettin. ent. Ztg. 29: 287-289.
- Doudoroff, M. 1938. Lactoflavin and bacterial luminescence. Enzymologia 5: 239-243.
- Doudoroff, M. 1942. Studies on the luminous bacteria. I. Nutritional requirements of some species, with special reference to methionine. J. Bact. 44: 451-460, 461-467.
- Doudoroff, M. 1942. Studies on the luminous bacteria. II. Some observations on the anaerobic metabolism of facultatively anaerobic species. J. Bact. 44: 461-467.

- Doyère, M. P. L. N. 1846. Sur la noctiluque miliarie. Inst. (Soc. math. phys. nat.) Paris, 14: No. 677, 428.
- Dubois, R. 1884, 1885. Note sur la physiologie des Pyrophores. C. R. Soc. Biol. Paris (Ser. 8) 1: 661-664; 2: 559-562, 1885.
- Dubois, R. 1885. Note sur la phosphorescence des poissons. Compt. Rend. Soc. Biol. Paris (Ser. 8) 2: 231-233.
- Dubois, R. 1885. Le vers luisant et l'éclairage des nids. Sci. Nat. Paris, No. 94.
- Dubois, R. 1886. Contribution à l'étude de la production de la lumière par les êtres vivants. Les elaterides lumineux. Bull. Soc. Zool. Fr. 11: 1-275.
- Dubois, R. 1886. De l'action de la lumière émise par les êtres vivants sur le rétiné et sur les plaques de gelatine bromure. C. R. Soc. Biol. Paris (Ser. 8) 3: 130.
- Dubois, R. 1886. De la fonction photogénique chez les Podures. C. R. Soc. Biol. Paris (Ser. 8) 3: 600-603.
- Dubois, R. 1886. De la fonction photogénique chez les myriapodes. C. R. Soc. Biol. Paris (Ser. 8) 3: 518-522, 523-525; 4, 6-8. Also in Rev. Sci. Paris (Ser. 3) 13: 509 (1887).
- Dubois, R. 1887. De la fonction photogénique dans les oeufs du lampyre. Bull. Soc. Zool. Fr. 12: 137-144, also in Bull. Ass. Franc. Sci. 1886: 155.
- Dubois, R. 1887, 1888. Fonction photogénique chez le *Pholas dactylus*. C. R. Acad. Sci. Paris 105: 690-692; and C. R. Soc. Biol. Paris (Ser. 8) 3: 564-565; 5: 451-453.
- Dubois, R. 1888. Mensuration, par la méthode graphique, des impressions lumineuses produits sur certains mollusques lamellibranches par des sources d'intensité et de longueurs d'onde différentes. C. R. Soc. Biol. Paris (Ser. 8) 5: 714-716.
- Dubois, R. 1888. Sur la rôle de la symbiose chez certains animaux marins lumineux. C. R. Acad. Sci. Paris 107: 502-504.
- Dubois, R. 1889. Remarques sur la physiologie et de l'anatomie du siphon de la *Pholade dactyle*. C. R. Soc. Biol. Paris (Ser. 9) 1: 521-523; C. R. Acad. Sci. Paris 109: 233, 320.
- Dubois, R. 1889. Les Microbes Lumineux. Extrait de Echo-Socs. vét., 1-24.
- Dubois, R. 1889. Sur la production de la lumière chez le *Pholas dactylus*. C. R. Soc. Biol. Paris 40: 451-453; 41: 611-614.
- Dubois, R. 1890. Nouvelles recherches sur la production de lumière par les animaux et par les végétaux. C. R. Acad. Sci. Paris 111: 363-366.
- Dubois, R. 1892. Anatomie et physiologie comparées de la *Pholade dactyle*. Ann. Univ. Lyon 2: (fascicle 2) 167 pp.
- Dubois, R. 1892. Sur la production de la phosphorescence de la viande par le *photobacterium sarcophilum*. Bull. Soc. Vaud. Sci. Nat. 27: 1-8. See Ann. Soc. Linn. Lyon 39.
- Dubois, R. 1893. Extinction de la luminosité du *photobacterium sarcophilum* par la lumière. (Ser. 9) 5: C. R. Soc. Biol., Paris, 160-161.
- Dubois, R. 1893. Sur le mécanisme de production de la lumière chez *Oryza barbarica* d'Algérie. C. R. Acad. Sci. Paris 117: 184-186.
- Dubois, R. 1896. Nouvelles recherches sur la production de la lumière par les êtres vivants. C. R. Soc. Biol. Paris 48: 995-996.
- Dubois, R. 1896. Les rayons X et les microbes lumineux. C. R. Soc. Biol. Paris 48: 479.
- Dubois, R. 1896. Physiological Light. Smithsonian. Inst. Ann. Rep. for 1895, 413-431, transl. from Rev. Gen. Sci. pur. appl., 5: 415-422, 429-534.

- Dubois, R. 1898. *Leçons de Physiologie Generale et Comparée*. Paris. 530 pp.
Production de la lumière et des radiations chimique par les êtres vivants.
Deuxieme part, pp. 301-528.
- Dubois, R. 1901. Sur le pouvoir éclairant et le pouvoir photochimique comparée
des bouillons liquides des photobacteries. Photographies obtenues par les
photobacteriacées, *Lampe vivante*. C. R. Soc. Biol. Paris 53: 133-134; also
Nature Paris 29: 293-294 (1901); and C. R. Acad. Sci. Paris, Aug. 27, 1900.
- Dubois, R. 1901. Nouvelles recherches sur la biophotogenèse. C. R. Soc. Biol.
Paris 53: 702-703.
- Dubois, R. 1904. *Lumière animale et lumière minerale*. C. R. Soc. Biol. Paris
56: 442-444, 621.
- Dubois, R. 1905. Response a M. Giesbrecht sur sa note "La Luminosite est-elle un
processus vital." C. R. Soc. Biol. Paris 58: 617-618, see 472-474.
- Dubois, R. 1907. De l'existence de certains principes fluorescents chez quelques
animaux invertébrés. C. R. Ass. Franç. Av. Sci. 35: 105, 470-472.
- Dubois, R. 1907. Mécanisme intime de la formation de la luciférine: analogies
et homologies des organes de Poli et la glande hypobranchiale des mollusques
purpurigènes. C. R. Soc. Biol. Paris 62: 850-852.
- Dubois, R. 1910. Sur la Biophotogenèse ou Production de la Lumière par les
êtres vivants. C. R. Ass. Franç. Av. Sci. 1-2.
- Dubois, R. 1911. Sur la fluorescence chez les insectes lumineux. C. R. Acad. Sci.
Paris 153: 208-210.
- Dubois, R. 1911. Nouvelles recherches sur la lumière physiologique chez
"*Pholas dactylus*." C. R. Acad. Sci. Paris 153: 690-692.
- Dubois, R. 1913. Mécanisme intime de la production de la lumière chez les
organismes vivants. *Ann. Soc. Linn. Lyon*, 60: 81-97.
- Dubois, R. 1913. Sur la nature et le development de l'organe lumineux du
Lampyre noctiluque. C. R. Acad. Sci. Paris 156: 730-732.
- Dubois, R. 1914. *La Vie et la Lumière*. Paris, 338 pp.
- Dubois, R. 1914. De la place occupée par la biophotogénèse dans la serie des phé-
nomènes lumineux. *Ann. Soc. Linn. Lyon* 61: 161-170.
- Dubois, R. 1914. Examen critique de la question de la biophotogénèse. *Ann. Soc.*
Linn. Lyon 61: 171-180.
- Dubois, R. 1914. Les animaux et les végétaux lumineux. Le secret de leur
fabrication et la lumière de l'avenir. C. R. Cong. Anglo-Franç. Ass. Franç. Av.
Sci. Paris, 12 pp.
- Dubois, R. 1916. Sur l'anatomie de la glande photogène de *Pholas dactylus*. A
propos d'un travail recent de M. J. Förster. *Ann. Soc. Linn. Lyon* 63: 9-13.
- Dubois, R. 1917. A propos de quelques recherches récentes de M. Newton Harvey
sur la biophotogénèse et du rôle important de la pré-luciférine. C. R. Soc. Biol.
Paris 80: 964-966 and C. R. Acad. Sci. Paris 165: 33.
- Dubois, R. 1917. Étude critique de quelques travaux récents relatif a la biophoto-
génèse. *Ann. Soc. Linn. Lyon*, 64: 65-118.
- Dubois, R. 1918. Sur la lumière physiologique (nouvelle repónse a M. Newton
Harvey). C. R. Soc. Biol. Paris 81: 484 and C. R. Acad. Sci. Paris 166: 578.
- Dubois, R. 1918. Nouvelles recherches sur la biophotogénèse. Synthèse naturelle
de la luciférine. C. R. Soc. Biol. Paris 81: 317-319.
- Dubois, R. 1919. Pseudo-cellules symbiotiques, anaérobies et photogènes. C. R.
Soc. Biol. Paris 82: 1016-19.

- Dubois, R. 1919. Réversibilité de la fonction photogénique par l'hydrogénase de la pholade dactyle. *C. R. Soc. Biol. Paris* 82: 840-842.
- Dubois, R. 1919. Symbiotes, Vacuolides, Mitochondries et Leucites. *C. R. Soc. Biol. Paris* 82: 473-475.
- Dubois, R. 1924. La pseudoluminescence et le rôle du tapis chez certains poissons. *C. R. Ac. Sci. Paris*, 178: 1030-1032 and *Nature*, Paris 52: 307-308.
- Dubois, R. 1924. Les Organismes Vivants Produisent de la Lumière Froide. Le secret de leur fabrication. *Nature*, Paris, No. 2604, 129-131.
- Dubois, R. 1928. Lumière (production de la) ou Biophotogénèse. In C. Richet's *Dictionnaire de Physiologie* (tome X) LAN-MO, 277-394.
- Dubois, R., et Aubert. 1884. Sur les propriétés de la lumière des pyrophores. *C. R. Acad. Sci.* 99: 477-479, and *C. R. Soc. Biol. Paris* (Ser. 8) 1: 602-604, 1884.
- Dubois, R., et P. Regnard. 1884. Note sur l'action des hautes pressions sur la fonction photogénique du Lampyre. *C. R. Soc. Biol. Paris* (Ser. 8) 1: 675-676.
- Duclaux, E. 1887. Revues et analyses sur les microbes phosphorescents. *Ann. Inst. Pasteur* 1: 489-495.
- Duges, M. 1837. *Lumbricus phosphoreus*, Annelides abranches sétigères. *Ann. Sci. Nat.* (Ser. 2) 8: 15-35.
- Dumeril, M. 1840. Indication d'observations anciennes relatives à la phosphorescence des Lombrics pendant une certaine saison. *C. R. Acad. Sci. Paris* 11: 747.
- Duncan, P. M. 1879. Some facts and theories about light emitting animals. *Pop. Sci. Rev. London*, 18: (N.S. 3): 225-242.
- Eaton, A. E. 1880. Sur la phosphorescence du *Caenis dimidiata*. *Trans. R. Ent. Soc. Lond. proc.* p. viii.
- Eaton, A. E. 1882. Luminous may-fly from Ceylon. *Trans. R. Ent. Soc. Lond. proc.* p. xiii.
- Edwards, F. W. 1924. A note on the New Zealand glow-worm (Diptera, Mycetophilidae). *Ann. Mag. Nat. Hist.* (Ser. 9) 14: 175-179.
- Edwards, F. W. 1934. The New Zealand glow-worm. *Proc. Linn. Soc. Lond.* 146: 3-10.
- Egorowa, A. A. 1929. Leuchtbakterien im Schwarzen und im Azowschen Meere. *Zbl. Bakt. Abt. 2*, 79: 168-173.
- Ehrenberg, C. G. 1830. Neue Beobachtungen über blutartige Erscheinungen in Aegypten, Arabien und Siberien, nebst einer Uebersicht und Kritik der früher bekannten. *Ann. Phys. Lpz.*, 18: 477-514.
- Ehrenberg, C. G. 1831. Ueber einen neuen, das Leuchten der Ostsee bedingenden lebenden Körper. *Ann. Phys. Lpz.*, 23: 147-151.
- Ehrenberg, C. G. 1873. Die das Funkeln und Aufblitzen des Mittelmeeres bewirkenden unsichtbaren kleinen Lebensformen. *Festschr. Ges. Naturf. Fr. Berlin*, 1873: 1-4.
- Ehrenberg, C. G. 1834. Das Leuchten des Meeres. *Abh. preuss. Akad. Wiss.*, 1834, 411-572. For review see *Ann. Mag. Nat. Hist.*, 1: 409-412, 1837.
- Ehrenberg, C. G. 1859. Über das Leuchten und über neue mikroskopische Leuchthiere des Mittelmeeres. *Mber. d. Preuss. Akad. Wiss.* 1859: 727-738, 791-793.
- Eigenmann, C. H., and R. S. Eigenmann. 1889. On the phosphorescent spots of *Porichthys margaritatus*. *West. Ann. Sci.* 6: 32-34; 132.
- Eijkmann, C. 1892. Lichtgebende Bacterien. *Geneesk. Tijdschr. Ned. Ind.* 32: 109-115, 435-441; *Zbl. f. Bact.* 12: 656, 1892.
- Linne, T. 1872. Bemerkungen über die Leuchtorgane von *Lampyrus splendidus*. *Arch. mikr. Anat.* 8: 652-653.

- Eliot, C. 1908. Reports on the marine biology of the Sudanese Red Sea. XI. Notes on a collection of nudibranchs from the red sea. *J. Linn. Soc. (Zool.)* 31: 86-122.
- Ellis, J. B. 1886. Phosphorescent fungi. *J. Mycol.* 2: 70-71.
- Elmhirst, R. 1912. Some observations on the glow-worm (*Lampyrus noctiluca*). *Zoologist (Ser. 4)* 16: 190-192, and *Knowledge* 35: 321, 1912.
- Emerson, G. A. 1935. Some effects of ether on bioluminescence in the lampyrid, *Photuris pennsylvanica*. *Proc. Soc. Exp. Biol. N.Y.*, 33: 36-40.
- Emerson, G. A., and M. J. Emerson. 1941. Mechanism of the effect of epinephrine on the fire-fly. *Proc. Soc. Exp. Biol. N.Y.*, 48: 700-703.
- Emery, C. 1884. Untersuchungen über *Luciola italica* L. *Z. wiss. Zool.* 40: 338-355.
- Emery, C. 1884. Recherches sur la *Luciola italica*. *Arch. ital. Biol.*, 5: 175-178; 7: 274-278, 1885.
- Emery, C. 1884. Intorno alle macchie splendenti della pelle nei pesci del genere *Scopelus*. *Mitteil. zool. Sta. Neapel* 5: 471-482. Also in *Arch. ital Biol.*, 5: 316-325.
- Emery, C. 1885. La luce della *Luciola italica*, asservata col microscopio. *Boll. Soc. ent. ital.* 17: 351-355 and 18: 406.
- Emery, C. 1886. Sur la lumiere des Lucioles (*Luciola italica*). *Arch. Sci. phys. nat.* 14: 272-275 and 16: 292.
- Emery, C. 1888. Das Leuchtorgan am Schwanz von *Scopelus Benoitii*. *Biol. Zbl.*, 8: 228-230; 10: 285-6, 1890.
- Emmerling, O. 1909. Hydrolyse der Meerleuchtinfusorien der Nordsee (*Noctiluca miliaris*). *Biochem. Z.* 18: 372-374.
- Enders, H. E. 1909. A study of the life history and habits of *Chaetopterus variopedatus*, Renier et Claparede. *J. Morph.* 20: 479-532.
- Englemann, T. W. 1863. Über die Vielzelligkeit von *Noctiluca*. *Zeit. wiss. Zool.* 12: 564-566.
- Epstein, T. 1930. Sur un bacterie lumineuse de la putrefaction provenant de l'aquarium du Musee Oceanographique de Monaco. *Bull. Inst. Océanogr. Monaco* No. 561, 4.
- Esterly, C. O. 1914. The vertical distribution and movements of the Schizopoda of the San Diego region. *Univ. Calif. Publ. Zool.* 13: 123-145.
- Evermann. 1838. *Lumbricus noctilucus*. *Utchen. Zapiski Kazan Univ.* 1838, 156-157. In Russian.
- Ewart, A. J. 1907. Note on the phosphorescence of *Agaricus (Pleurotus) canescens* Müll. *Vict. Nat.* 13: 174.
- Eymers, J. G. and K. L. Van Schouwenburg. 1936. On the luminescence of bacteria. I. A quantitative study of the spectrum of the light emitted by *Photobacterium phosphoreum* and by some chemiluminescent reactions. *Enzymologia* 1: 107-119.
- Eymers, G., and K. L. Van Schouwenburg. 1937. On the luminescence of bacteria. II. Determination of the oxygen consumed in the light emitting process of *Photobacterium phosphoreum*. *Enzymologia* 1: 328-340.
- Eymers, G., and K. L. Van Schouwenburg. 1937. On the luminescence of bacteria. III. Further quantitative data regarding spectra connected with bioluminescence. *Enzymologia* 3: 235-241.
- Eyring, H. and J. L. Magee. 1942. Application of the theory of absolute reaction rates to bacterial luminescence. *J. Cell. Comp. Physiol.* 20: 169-177.

- Eyring, H., R. Lumry, and J. W. Woodbury. 1949. Some applications of modern rate theory to physiological systems. *Rec. Chem. Progr. Summer Issue*, 100-103.
- Fabre, J. H. 1855. Recherches sur la cause de la phosphorescence d'Agaric de l'Olivier. *Ann. Sci. Nat. (Ser. 4)* 4: 179-197.
- Fabre, H. 1914. The glow-worm. The first user of anaesthetics. *Century Mag.* 87: 105-112. Also in "The wonders of instinct," 1918, N.Y., pp. 268-288.
- Fage, L. 1934. Sur la présence d'organes lumineux chez les Amphipodes pélagiques. *C. R. Acad. Sci. Paris* 198: 1631-1633.
- Falger, F. 1908. Untersuchungen über das Leuchten von Acholoë astericola. *Biol. Zbl.* 28: 641-649.
- Fanzago, E. 1881. Sulla secrezione ventrale del *Geophilus gabrielis*. *Atti. Ist. Veneto* 7: 641-646.
- Farghaly, A. H. 1950. Factors influencing the growth and light production of luminous bacteria. *J. Cell. Comp. Physiol.* 36: 165-184.
- Faure-Fremiet, E. 1910. Etudes sur les mitochondries des protozoaires, et des cellules sexuelles. *Arch. Anat. micr.* 11: 457-648.
- Faure-Fremiet, E. 1910. Le tentacle de la *Noctiluca miliaris*. *Bull. Soc. zool. Fr.* 35: 8.
- Fejgin, B. 1926. Études sur les microbes marins. Étude sur une bactérie lumineuse. *Bull. Inst. oceanogr. Monaco* No. 471, 1-4.
- Fennell, J. 1835. The larva of the glow-worm differs from the perfect insect. *Loudon's Mag. Nat. Hist. (Ser. la)* 8: 625-626.
- Ferguson, E. W. 1925. Description of a new species of mycetophilidae (diptera) with luminous larvae. *Proc. Linn. Soc. N.S. W.* (2) 50: 487-488.
- Fischer, A. 1900. The structure and functions of bacteria. Oxford, p. 30; 63.
- Fischer, B. 1887. Bacteriologische Untersuchungen auf einer Reise nach West Indien. II. Über einen lichtenwickelnden, in Meerswasser gefunden Spaltpilz. *Z. Hyg. Infektr.* 2: 54-95.
- Fischer, B. 1888. Bakterien Wachstum bei 0° so wie über das Photographieren von Kulturen leuchtender Bakterien in ihren eigenen Lichte. *Zbl. Bakt.* 4: 89-92.
- Fischer, B. 1888. Ueber einen neuen lichtenwickelnden Bacillus. *Zbl. Bakt.* 3: 105-108, 137-141.
- Fischer, B. 1894. Die Bakterien des Meeres. *Ergebn. Plank. Exped. Humboldt Stiftung*, 4: 1-83.
- Floericke, Kurt. 1908. Nächtlche Waldbeleuchtung. *Kosmos. Stuttgart* 5: 240-242.
- Foa, G., and A. R. Chiapella. 1903. Ricerche sopra un nuovo microorganismo fosforescente. *Sperimentale Arch. d. biol. Firenze* 57: 274-309.
- Forbes, E. 1848. A monograph of the naked-eyed Medusae with figures of all the species. London (Ray. Soc.) 104 pp. Phosphorescence, pp. 11-14.
- Forster, J. 1887. Ueber einige Eigenschaften leuchtender Bakterien. *Zbl. Bakt.* 2: 337-340.
- Forster, J. 1892. Über die Entwicklung von Bakterien bei niedriger Temperatur. *Zbl. f. Bakt.* 12: 431-436.
- Förster, J. 1914. Über die Leuchtorgane und das Nervensystem von *Pholas dactylus*. *Z. wiss. Zool.* 109: 349-393.
- Forsyth, R. W. 1910. The spectrum of bacterial luminosity. *Nature, Lond.* 83: 7.
- Frankel, C. 1889. Die Einwirkung der Kohlensäure auf die Lebensfähigkeit von Mikroorganismen. *Z. Hyg. Infekt. Kr.* 5: 332-362.

- Frankland, P. 1898. The action of bacteria on the photographic plate. *Zbl. Bakt.* (Abt. 1) 24: 609-612.
- Franklin, C. L. 1900. Phosphorescence in deep-sea animals. *Science N.S.* 11: 954.
- Fraser, C. McL. 1915. The swarming of *Odontosyllis*. *Trans. Roy. Soc. Can.* 9: Section 4, 43-49.
- French, J. W. 1925. Bioluminescence. *Nature, Lond.* 115: 944-945.
- French, J. W. 1937. Phosphorescence of the sea. *Nature Lond.* 139: 804-805.
- Friedberger, E., and H. Doepner. 1907. Über den Einfluss von Schimmelpilzen auf die Lichtintensität in Leuchtbakterienkulturen etc. *Zbl. Bakt.* (Abt. 1) 43: 1-7.
- Friend, H. 1893. Luminous earthworms. *Nature, Lond.* 47: 462-463.
- Friend, H. 1897. Earthworm studies. III. Phosphorescence and luminosity. *Zoologist* (Ser. 4) 1: 304-311.
- Friend, H. 1910. Luminous worms in Ireland. *Irish Nat.* 19: 105-107.
- Friend, H. 1913. Annelid hunting in Notts. 3rd paper. *Rep. Notts. Nat. Soc.* 1912-13: 24-26.
- Friend, H. 1919. Luminous worms. *Nature, Lond.* 103: 446.
- Friend, H. 1924. The story of British annelids. Chap. VII Phosphorescence and Luminosity, London.
- Fripp, H. 1866. On the light emitting apparatus of the Glowworm. *Pop. Sci. Rev.* 5: 314-326.
- Fuchs, S. 1891. Einige Versuche an der Leuchtorganen von *Elater noctilucus*. *Zbl. Physiol.* 5: 321-325.
- Fuhrmann, F. 1932. Studien zur Biochemie der Leuchtbakterien. I. Der Einfluss von Na und K Chlorid und Bromid auf die Lichtentwicklung von *Photobacillus radians*. II. Der Einfluss von Zuckern mit NaCl. *Mh. Chem.* 60: 69-105; 414-430.
- Fujiwara, T. 1935. On the light production and luminous organs in a Japanese Chaetopterid, *Mesochaetopterus japonicus*, Fujiwara. *J. Sci. Hiroshima Univ.* 3: 185-192.
- Fulton, B. B. 1939. Lochetic luminous dipterous larvae. *J. Elisha Mitchell Sci. Soc.* 55: 289-293.
- Fulton, B. B. 1941. A luminous fly-larva with spider traits (Diptera, Mycetophilidae). *Ann. Ent. Soc. Amer.* 34: 289-302.
- Fulton, B. B. 1941. Flies that trap spiders. *Frontiers* 5: 149-152.
- Gadeau de Kerville, H. 1881. Les insectes phosphorescents. Rouen, 55 pp.
- Gadeau de Kerville, H. 1887. Les insectes phosphorescents. Notes complementaire et bibliographie generale. (Anatomie, Physiologie et Biologie.) Rouen. 133 pp.
- Gadeau de Kerville, H. 1890. Les Vegetaux et les Animaux Lumineux. Rouen, 327 pp. In german, Leipzig, 1893.
- Gahan, J. C. 1907. A remarkable luminous insect from Brazil. *Zoologist* (Ser. 4) 11: 277.
- Gahan, J. C. 1908. Lampyridae from Ceylon. *Trans. R. ent. Soc. Lond.* 1908 Proc., p. XLVIII; and *Entomologist*, 41: 205 (1908).
- Gahan, J. C. 1924. Living fireflies from Argentina. *Trans. R. ent. Soc. Lond.* p. V.
- Gallardo, T. W. 1908. A case of phosphorescence as a mating adaptation. *Sci. Sci. Math.* May.

- Galloway, T. W., and P. S. Welch. 1911. Studies on a phosphorescent Bermudan annelid, *Odontosyllis enopla* Verrell. *Trans. Amer. micr. Soc.* 30: 13-39.
- Galtsoff, P. S. 1948. Red tide; progress report on the investigations of the cause of the mortality of fish along the west coast of Florida. U.S. Dept. Inter. Fish Wildlife Serv. Spec. Sci. Rep. No 46. 44 pp.
- Galtsoff, P. S. 1949. The mystery of the red tide. *Sci. Mon.* 68: 108-117.
- Gardiner, J. S., and C. F. Cooper. 1907. Description of the Percy Sladen Trust Expedition to the Indian Ocean in 1905. *Trans. Linn. Soc. Lond. (Ser. 2)* 12: 1-56.
- Garman, S. 1899. Reports on an exploration off the west coast of Mexico, Central and South America, and off the Galapagos Islands, in charge of Alexander Agassiz, by the U.S. Fish Commission steamer *Albatross*, during 1891. The fishes. *Mem. Narr. Mus. comp. Zool.* 24: 431 pp.
- Gates, F. C. 1917. Synchronism in the flashing of fire-flies. *Science* 46: 314.
- Gates, G. E. 1925. Note on luminescence in the earthworms of Rangoon. *Rec. Indian Mus.* 27: 6, 471-473.
- Gates, G. E. 1944. Note on luminescence in some Allahabad earthworms. *Curr. Sci.* 13: 131-132.
- Gatti, M. 1899. Ricerche sugli organi biofotogenetici dei pesci. Parte 2. Organi di tipo elettrico. Parte 3. Sviluppo degli organi dei due tipi. *R. C. Accad. Lincei*, 8: 81-87.
- Gatti, M. 1904. Richerche sugli organi luminosi dei pesci. *Annali di Agricoltura. Lav. eseg. nella R. Sta. di piscicoltura*, 65 pp.
- Gazagnaire, J. 1888. La phosphorescence chez les Myriapods. *Bull. Soc. Zool. Fr.* 13: 182-186.
- Gazagnaire, J. 1890. La phosphorescence chez les myriapods de la famille des Geophilidae—Epoque et conditions physiologiques de l'apparition de la phosphorescence. *Mem. Soc. Zool. Fr.* 3: 136-146.
- Geipel, E. 1915. Beiträge zur Anatomie der Leuchtorgane tropischer Käfer. *Z. wiss. Zool.* 112: 239-290.
- George, H. 1851. Note on the phosphorescence of *Goërius olens*, Müller. *Trans. R. ent. Soc. Lond. N.S.* 1, Proc., 117.
- Gerretsen, F. C. 1915. Die Einwirkung des Ultravioletten Lichtes auf die Leuchtbakterien. *Zbl. Bakt. (Abt. 2)* 44: 660-661.
- Gerretsen, F. C. 1920. Über die Ursachen des Leuchtens der Leuchtbakterien. *Zbl. Bakt. (Abt. 2)* 52: 353-373.
- Gerretsen, F. C. 1922. Einige Notizen über der Leuchten des Javanischen Leuchtkäfers (*Luciola vittata* Cast.). *Biol. Zbl.* 42: 1-9.
- Getsel, D. 1934. I microbi della glandola nidamentale accessoria in *Sepia officinalis*. *Priva nota. Arch. Zool. (ital.) Napoli* 20: 33-43.
- Gianferrari, L. 1922. Organi luminosi a bacteri nei pesci. *Natura, Padova* 13: 82-83.
- Giard, A. 1887. Sur un nouveau genre de *Lombricus phosphorescens* et sur l'espèce type de ce genre, *Photodrillus phosphoreus* Dugès. *C. R. Acad. Sci. Paris* 105: 872-874.
- Giard, A. 1889. On the phosphorescent infection of the Talitri and other crustaceans. *Ann. Mag. nat. Hist. (6)* 4: 476-478.
- Giard, A. 1890. Les animaux et les vegetaux Lumineux. *Rev. Sci., Paris* 45: 29-30.
- Giard, A., and A. Billet. 1889. Observations sur la maladie phosphorescente des

- Talitres et autres crustacés. C. R. Soc. Biol. Paris 41: 593-597 and C. R. Acad. Sci. Paris 109: 503.
- Giard, A., and A. Billet. 1890. Nouvelles recherches sur les bacteries lumineuses Pathogènes. C. R. Soc. Biol., Paris (Ser. 9), 2: 188-191 (1890).
- Giesbrecht, W. 1892. Pelagischen Copepoden des Golfes von Neapel. 830 pp. Also Flora Fauna Golf Neapel, 19.
- Giesbrecht, W. 1895. Ueber das Leuchten der pelagischen Copepoden und das tierische Leuchten im allgemeinen. Mitt. Zool. Sta. Neapel 11: 631-694.
- Giesbrecht, W. 1896. Ueber den Sitz der Lichtentwicklung in den Photosphären der Euphausiiden. Zool. Anz. 19: 486-490.
- Giesbrecht, W. 1905. La luminosité est-elle un processus vital? C. R. Soc. Biol. Paris 58: 472-474. Réponse par Raphael Dubois, 617-619.
- Giese, A. C. 1941. Effects of ultraviolet radiations on luminescence and respiration of *Achromobacter Fischeri*. J. cell. comp. Physiol. 17: 203-220.
- Giese, A. C. 1943. Studies on the nutrition of dim and bright variants of a species of luminous bacteria. J. Bact. 46: 323-331.
- Giese, A. C. 1945. The action of azide on luminescence, respiration and growth of the luminous bacteria. J. cell. comp. Physiol. 26: 75-86.
- Giese, A. C. 1946. The effect of boric acid on the respiration and luminescence of *Achromobacter fisheri*. Anat. Rec. 96: 89.
- Giese, A. C., and A. M. Chase. 1940. The effects of cyanide on *Cypridina luciferin*. J. cell. comp. Physiol. 16: 237-246.
- Giglioli, E. H. 1870. La fosforescenza del mare. Note pelagiche ed osservazioni fatte durante un viaggio circumnavigazione 1865-68 Colla descrizione di due nuove noctiluche. Atti Accad. Torino 5: 485-505.
- Gilbert, C. H. 1903. Deep sea fishes: The aquatic resources of the Hawaiian Islands; Part II, Section 2. Bull. U.S. Fish Comm. 23: 577-713.
- Gilbert, C. H. 1908. The lantern fishes. Mem. Harv. Mus. comp. Zool. 26: 218-237.
- Gilbert, C. H. 1915. Fishes collected by U.S. Fisheries steamer "Albatross" in 1904. Proc. U.S. nat. Mus. 48: 305-380.
- Gilchrist, J. D. F. 1919. Luminosity and its origin in a South African earth-worm (*Chitota* sp.). Trans. roy. Soc. S. Afr. 7: 203-212.
- Gill, Theo. 1909. Angler Fishes; their kind and ways. Rep. Smithson. Instn. 1908, 565-615. See also Nat. geogr. Mag. 21: 453-456, 1910.
- Gimmerthal, B. A. 1829. Observations sur la metamorphosis de quelques Dipteres de la famille des Muscides, et sur la phosphorescence d'une chenille de Noctuelle (*Noctua occulta*, L.). Bull. Soc. Imp. Nat. Moscow 1: 136-141. Also in: Bull. Sci. Nat. 26: 101; and: Ann. Soc. ent. Fr. (1832); 424.
- Girard, M. 1873. Les Taupins lumineux. Nature, Paris 1: 337-339.
- Göbel, F. 1824. Leuchtende Entwicklung der Kohlensäure. J. Chem. Phys. 40. (N.s.) 10: 257-279.
- Goggia, P. 1910. Phénomènes lumineux dans la serie animale. Cosmos, Paris (N.s.) 63: 270-274, 299-303.
- Goldschmidt, R. B. 1948. Glow-worms and evolution. Rev. Sci., Paris 86: 607-612.
- Goode, G. B., and T. H. Bean. 1895. Oceanic Ichthyology, a treatise on the deep-sea and pelagic fishes of the world. Gov. Printing Office, Washington, D.C.
- Goor, A. C. J. van. 1918. Die cytologie von *Noctiluca miliaris* im Lichte der neuen Theorien über der Kernbau der Protisten. Arch. Protistenk. 39: 147-208.

- Gordon, I. 1935. On new or imperfectly known species of the Crustacea *Macrura*. J. linn. Soc. (Zool.) 39: 307-318.
- Gorham, H. S. 1880. On the structure of the *Lampyridae* with reference to their phosphorescence. Trans. R. ent. Soc. Lond., 1880, 63-67.
- Gorham, F. G. 1904-1905. Die lichterzeugenden Bakterien. Zbl. Bakt. (Abt. II), 13: 227-228.
- Gosse, P. H. 1848. On the insects of Jamaica. Ann Mag. nat. Hist. (Ser. 2), 1: 197-202.
- Gosse, P. H. 1853. A naturalist's rambles on the Devonshire coast. London, 451 pp. Luminosity of sea and Noctiluca, pp. 250-253.
- Gounelle, E. 1886. Note sur la Fulgora lanternaria. Ann. Soc. ent. Fr. (Ser. 6), 6: bull. 100-101.
- Gourret, P. 1883. Sur les Peridiens du Golfe de Marseille. Ann. Mus. Hist. nat. Marseille, Zool. 1: 1-144.
- Goutaland, M. 1936. Sur la phosphorescence de "Clytocybe olearia." Bull. mens. Soc. linn. Lyon 5: 142-143.
- Graves, R. J. 1834-1835. Lectures on Phosphorescence. Lond. Med. Surg. J. 6: 705-710.
- Graves, R. J. 1863. Phosphorescence. Stud. Physiol. Med., London, pp. 47-54.
- Greiff, R. 1879. Über pelagische Anneliden von der Küste der Kanarischen Inseln. Z. wiss. Zool. 32: 255-283.
- Greiff, R. 1882. Ueber die rosettenförmigen Leuchtorgane der Tomopteriden und zwei neue Arten von Tomopteris. Zool. Anz. 5: 384-387; Abs. in J. R. micr. Soc. 2 (Ser. 2), 780, 1882.
- Greiff, R. 1885. Ueber die pelagische Fauna an den Küsten der Guinea-Inseln. Z. wiss. Zool. 42: 432-458.
- Green, E. E. 1911. On the occasional luminosity of the beetle "*Harmatelia bilinea*." Spolia zeylan. 7: 212-214.
- Green, E. E. 1913. On some luminous coleoptera from Ceylon. Trans. R. ent. Soc. Lond., 1912, 717-719.
- Green, E. E. 1915. Giant Glow-worm. (*Lamprophorus tenebrosus*.) Ent. Soc. Lond. Exhibit. Knowledge 28: 359.
- Greene, C. W. 1899. The phosphorescent organs in the toad fish. *Porichthys notatus* Girard. J. Morph. 15: 667-696.
- Greene, C. W., and H. H. Greene. 1924. Phosphorescence of *Porichthys notatus*, the California Singing Fish. Amer. J. Physiol. 70: 500-507.
- Griffin, A. W. 1887. Noctiluca miliaris. J. Micr. Nat. Sci. 6: 7-15.
- Grimpe, G. 1930. Echinodermata (Stachelhautre) in Tabul. Biol., Berlin 6: 499-501.
- Grimpe, G., and H. Hoffmann. 1921. Ueber die postembryonalentwicklung von *Histioteuthis* und über ihre sogenannten "Endorgane." Arch. Naturgesch. 87: Abt. A, Heft 12, 179-219.
- Grimpe, G., and H. Hoffmann. 1930. Mollusca. Production von Licht. Tabul. Biol., Berl. 6: 462-464.
- Griner, A. M., A. A. Lytell, and H. Kerstein. 1945. Luminometer for measuring bacterial luminescence. Rev. sci. Instrum. 16: 10-14.
- Grinfeld, R. 1944. Contribucion al estudio del espectro de la luz de la luciernagas. Contrib. Fac. Ciencias Fisicomatemat. La Plata, Argentine, 3: 447-461.
- Gross, F. 1934. Zur Biologie und Entwicklungsgeschichte von *Noctiluca miliaris*. Arch. Protistenk. 83: 178-196.

- Gross, F. 1937. Notes on the culture of some marine plankton organisms. *J. Mar. biol. Assoc. U.K.* 21: 753-768.
- Grotthuss, T. de. 1807. Sur la combinaisons du phosphore avec les metaux et leurs oxides par la voie humide etc. *Ann. Chim.* 64: 19-41.
- Grube, A. E. 1861. *Ausflug nach Triest und dem Quarnero.* 175 pp. Berlin, p. 79.
- Grube, A. E. 1864. *Die Insel Lussin und ihre Meeresfauna.* Breslau.
- Gudger, E. W., and L. L. Mowbray. 1927. The oilfish, *Ruvettus pretiosus* at Bermuda. *Science* 65: 145-146.
- Guéguen, M. F. 1907. Recherches biologiques et anatomiques sur le *Xylaria Hypoxylon*. *Bull. Soc. mycol. Fr.* 23: 186-217.
- Günther, A. 1880. An introduction to the study of fishes. Chap. XXI on the fishes of the deep sea, 296-311. Edinburgh, 720 pp.
- Günther, A. 1887. Report in the deep-sea fishes collected by H. M. S. Challenger during the year 1873-76. *Challenger Reports, Zoology*, 22: 335 pp.
- Guilding, L. 1834. Notes on luminous insects, chiefly of the West Indies; on luminous meteors; on ignes fatui; on the luminousness of the sea, etc. *Mag. nat. Hist.* 7: 579-583.
- Gunter, G., R. H. Williams, C. C. Davis, and F. G. Walton-Smith. 1948. Catastrophic mass mortality of marine animals and coincident phytoplankton bloom on the west coast of Florida, November 1946 to August 1947. *Ecol. Monogr.* 18: 309-324.
- Guppy, H. B. 1882. Note on the pearly organs of *Scopelus*. *Ann. Mag. nat. Hist.* 9: (Ser. 5) 202-204.
- Guyot, R. 1927. *Mycelium lumineux d l'Armillaire.* *C. R. Soc. Biol. Paris*, 96: 114; also *Feuill. Nat.* 21: 165-168, 1925.
- Haase, E. 1886. Ein neuer Phengodes. *Ent. Nachr.* 12: 218-219.
- Haase, E. 1888. Zur Kenntniss von Phengodes. *Dsch. ent. Z.* 32: 145-167.
- Haase, E. 1889. Ueber das Leuchten der Myriopoden. *Tagebl. 61. Vers. Deutsche Naturforscher. u. Aerzte, Köln.* 48-49.
- Hada, Y. 1939. Hydrographical observations and plankton studies of some brakish water lakes on the Okhotsk Sea Coast of Hokkaido in winter. *Trans. Sapporo nat. Hist. Soc.* 16: 147-174.
- Haddon, K. 1915. On the methods of feeding and the mouth-parts of the larva of the Glow-worm (*Lampyrus noctiluca*). *Proc. Zool. Soc. Lond.* (March) 77-82. Also in *Knowledge*, 38: 216.
- Haga, M. 1942. Experimental studies on the penetration of the luminous bacteria through the shell of the hen's egg. *Seiikai*, 62: 693-698. *Sei-i-Kwai med. J.* In Japanese.
- Haga, M. 1944. Studies of the antagonistic action of the luminous bacteria against the *Staphylococcus*. *Sei-i-Kwai med. J.* 63: 53-62. In Japanese.
- Hagen, H. 1853. *Alteste Nachricht über das Leuchten der Fulgora lanternaria.* *Stettin. ent. Ztg.*, 14: 55-56.
- Hagen, H. 1865. On the luminosity of *Fulgora lanternaria*. *Ent. Month. Mag.* 1: 250-251.
- Hagen, H. A. 1873. Notes on the Ephemeridae. *Trans. R. ent. Soc. Lond.* 399.
- Hagitani, A. 1941-47. Bioluminescenz. *In Progr. Org. Chem.* X. Enzyme Chemistry: 403-437. In Japanese.
- Hamada, M. 1940. Physiologisch morphologische Studien über *Armillaria mellea*

- (Vahl) Quél., mit. besonderer Rücksicht auf die Oxalsäurebildung. Jap. J. Bot. 10: 387-463.
- Hancock, J. 1834. No title. Note upon the luminosity of *Fulgora lanternaria* L. Trans. R. ent. Soc. Lond. 1: proc. xxxii. Also in Proc. zool. Soc. Lond. part 2: 19, 1834.
- Handrick, K. 1901. Zur Kenntniss des Nervesystems und der Leuchtorgane von *Argyropelecus hemigymnus*. Zoologica, Stuttgart 13 (Heft 32), 1-68.
- Handschin, E. 1921. Leuchtende Collembolen. Verh. Schweiz. Naturforsch. Ges. 101 Jahrsvers. 2 teil, 222-223.
- Handschin, E. 1926. Collembola in Biol. Tiere Dtschl. Lief. 20 (Teil 25), 19.
- Haneda, Y. 1938. On the luminescence of the deep-sea fish *Malacocephalus laevis* (Lowe). Jap. J. Physiol. 3: 318-326; also in Jap. J. Med. Sci. III. Biophysics. 5: 355-366.
- Haneda, Y. 1938. Luminous fishes from southern seas. Kagaku Nanyo 1: 21-27; also Zool. Mag. Tokyo 51: 105. In Japanese.
- Haneda, Y. 1939. A few observations on the luminescent fungi of Micronesia. Kagaku Nanyo 1: 116-128. In Japanese.
- Haneda, Y. 1939. Luminosity of *Rocellaria grandis* (DESHAYES) (Lamelli-branchia). Kagaku Nanyo, 2: 36-39. In Japanese.
- Haneda, Y. 1939. Luminescence of the living matters. Kagaku, 9: 253-259. In Japanese.
- Haneda, Y. 1939. The terrestrial luminescent animals and plants in Palau and Yap Islands. Kagaku Nanyo 2: 88-93. In Japanese.
- Haneda, Y. 1940. Phenomena of bioluminescence. Seirigaku Shidoshu 5: 18-31. In Japanese.
- Haneda, Y. 1940. On the luminescence of the fishes belonging to the family Leionathidae of the tropical Pacific. Palao Trop. biol. Sta. Stud. 2: 29-39.
- Haneda, Y. 1941. Note on the light production in the Schan-Schan. Kagaku Nanyo 3: 181-183. In Japanese.
- Haneda, Y. 1942. Further investigation upon luminescent fungi. Kagaku Nanyo 4: 49-63. In Japanese.
- Haneda, Y. 1943. On the photogenous organ of *Anomalops katoptron*, a luminous fish. Kagaku Nanyo 5: 81-88. In Japanese.
- Haneda, Y. 1946. A luminous land snail, *Dykia striata*, found in Malaya. Seibutu 294-298. In Japanese.
- Haneda, Y. 1950. Star-worm in Singapore. Shin Konchu 3: 2-5. In Japanese.
- Haneda, Y. 1950. *Harpodon nehereus*, a non-luminous fish. Pacif. Sci. 4: 135-138.
- Haneda, Y. 1950. Luminous organs of fish which emit light indirectly. Pacif. Sci. 4: 214-227.
- Haneda, Y., and N. Kumagai. 1939. The luminous material of *Pontodrilus matsushimensis*, Izuka. Jap. J. Physiol. 4: 328-333. In Japanese.
- Haneda, Y., K. Takase and N. Kumagaya. 1940. Spectroscopical study of the living light. Jap. J. Physiol. 5: 307-317. In Japanese.
- Hankel, W. G. 1862. Notiz über phosphorische Leuchten des Fleisches. Ann. Phys. (Ser. 4) 25: 62-70; also Ber. dtsh. chem. Ges. 13: 5-12, 1861; and J. prakt. Chem. 83: 153-161, 1861.
- Hanna, W. F. 1938. Notes on *Clitocybe illudens*. Mycologia 30: 379-384.
- Hansen, H. J. 1903. On the crustaceans of the genera *Petalidium* and *Sergestes*

- from the "Challenger" with an account of luminous organs in *Sergestes challengerii* n. sp. Proc. zool. Soc. Lond. 1: 52-79.
- Harker, Allen. 1888. On a luminous oligochaete (*Enchytraeus*). Rep. Brit. Ass. Manchester, 1887, 767.
- Harms, J. W. 1928. Bau und Entwicklung eines eigenartigen Leuchtorgans bei *Equula spec.* Zeit. wiss. Zool. 131: 157-179.
- Hartig, T. 1855. Über das Leuchten des weissfaulen Holzes. Bot. Ztg. 13: 148-149.
- Harvey, E. B. 1917. A physiological study of specific gravity and luminescence in *Noctiluca* with special reference to anaesthesia. Publ. Carneg. Instn. No. 251, 235-253; also Proc. Nat. Acad. Sci. 3: 15-16.
- Harvey, E. N. 1913. The temperature limits of phosphorescence of luminous bacteria. Biochem. Bull. 2: 456-457.
- Harvey, E. N. 1914. On the chemical nature of the luminous material of the fire-fly. Science 40: 33-34.
- Harvey, E. N. 1915. The effect of certain organic and inorganic substances upon light production by luminous bacteria. Biol. Bull. Wood's Hole 29: 308-312.
- Harvey, E. N. 1915. Studies on light production by luminous bacteria. Amer. J. Physiol. 37: 230-240. (I of "Studies.")
- Harvey, E. N. 1915. Experiments on the nature of photogenic substance in the firefly. J. Amer. Chem. Soc. 37: 396-401.
- Harvey, E. N. 1916. The mechanism of light production in animals. Science 44: 208-209.
- Harvey, E. N. 1916. The light-producing substances photogenin and photophelein, of luminous animals. Science 44: 652-654.
- Harvey, E. N. 1916. Studies on bioluminescence. II. On the presence of luciferin in luminous bacteria. Amer. J. Physiol. 41: 449-454.
- Harvey, E. N. 1917. What substance is the source of the light in the firefly? Science 46: 241-243.
- Harvey, E. N. 1917. The chemistry of light production in luminous organisms. Publ. Carneg. Instn. No. 251, 171-234.
- Harvey, E. N. 1917. An instance of apparent anesthesia of a solution. Amer. J. Physiol. 42: 606.
- Harvey, E. N. 1917. Studies on bioluminescence. IV. The chemistry of light production in a Japanese ostracod crustacean, *Cypridina hilgendorffii*, Müller. Amer. J. Physiol. 42: 318-341.
- Harvey, E. N. 1917. Studies on bioluminescence. V. The chemistry of light production by the fire-fly. Amer. J. Physiol. 42: 342-348.
- Harvey, E. N. 1917. Studies in bioluminescence. VI. Light production in a Japanese pennatulid, *Cavernularia haberi*. Amer. J. Physiol. 42: 349-358.
- Harvey, E. N. 1918. Studies on bioluminescence. VII. Reversibility of the photogenic reaction in *Cypridina*. J. gen. Physiol. 1: 133-145.
- Harvey, E. N. 1919. Studies on bioluminescence. IX. Chemical nature of *Cypridina* luciferin and *Cypridina* luciferase. J. gen. Physiol. 1: 269-293.
- Harvey, E. N. 1919. Studies on bioluminescence. X. Carbon dioxide production during luminescence of *Cypridina* luciferin. J. gen. Physiol. 2: 133-135.
- Harvey, E. N. 1919. Studies on bioluminescence. XI. Heat production during luminescence of *Cypridina* luciferin. J. gen. Physiol. 2: 137-143.
- Harvey, E. N. 1920. Studies on bioluminescence. XII. The action of acid and of light in the reduction of *Cypridina* oxyluciferin. J. gen. Physiol. 2: 207-213.

- Harvey, E. N. 1920. Is the luminescence of Cypridina an oxidation. *Amer. J. Physiol.* 51: 580-587.
- Harvey, E. N. 1920. *The Nature of Animal Light*. Philadelphia, 182 pp.
- Harvey, E. N. 1921. *Animal Light*. *Trans. Illum. Engng. Soc.* 16: 319-330.
- Harvey, E. N. 1921. Studies on bioluminescence. XIII. Luminescence in the Coelenterates. *Biol. Bull. Wood's Hole* 41: 280-287.
- Harvey, E. N. 1922. Studies on bioluminescence. XIV. The specificity of luciferin and luciferase. *J. gen. Physiol.* 4: 285-295.
- Harvey, E. N. 1922. The permeability of cells for oxygen and its significance for the theory of stimulation. *J. gen. Physiol.* 5: 215-222.
- Harvey, E. N. 1923. The production of light by the fishes, Photoblepharon and Anomalops. *Publ. Carneg. Instn. No. 312*, 43-60. Also in *Science* 53: 314-315, 1921.
- Harvey, E. N. 1923. Animal luminescence. *J. Franklin Inst.* 196: 31-44.
- Harvey, E. N. 1923. Studies on bioluminescence. XV. Electroreduction of oxyluciferin. *J. gen. Physiol.* 5: 275-284.
- Harvey, E. N. 1923. The minimum concentration of luciferin to give a visible luminescence. *Science* 57: 501-503.
- Harvey, E. N. 1924. Recent advances in bioluminescence. *Physiol. Rev.* 4: 639-671.
- Harvey, E. N. 1924. Studies on bioluminescence. XVI. What determines the color of the light of luminous animals? *Amer. J. Physiol.* 70: 619-623.
- Harvey, E. N. 1924. Neue Versuche uber Bioluminescence. *Naturwissenschaften* 9: 165-169.
- Harvey, E. N. 1925. Luminous fishes of the Banda Sea. *Nat. Hist., N.Y.* 25: 353-356.
- Harvey, E. N. 1925. Studies on bioluminescence. XVII. Fluorescence and inhibition of luminescence in ctenophores by ultraviolet light. *J. gen. Physiol.* 7: 331-339.
- Harvey, E. N. 1925. The effects of light on luminous bacteria. *J. gen. Physiol.* 7: 687-691.
- Harvey, E. N. 1925. The inhibition of Cypridina luminescence by light. *J. gen. Physiol.* 7: 679-685.
- Harvey, E. N. 1925. The effects of light on luminous bacteria. *J. gen. Physiol.* 7: 687-691.
- Harvey, E. N. 1925. The total luminous efficiency of luminous bacteria. *J. gen. Physiol.* 8: 89-108.
- Harvey, E. N. 1926. Luminous bacteria, the smallest lamps in the world. *Sci. Amer.* 315: 414-416.
- Harvey, E. N. 1926. Additional data on the specificity of luciferin and luciferase, together with a general survey of this reaction. *Amer. J. Physiol.* 77: 548-554.
- Harvey, E. N. 1926. Bioluminescence and fluorescence in the living world. *Amer. J. Physiol.* 77: 555-561.
- Harvey, E. N. 1926. Further studies on the inhibition of Cypridina luminescence by light, with some observations on methylene blue. *J. gen. Physiol.* 70: 101-110.
- Harvey, E. N. 1926. On the inhibition of animal luminescence by light. *Biol. Bull. Wood's Hole* 51: 85-88.

- Harvey, E. N. 1926. Oxygen and luminescence, with a description of methods for removing oxygen from cells and fluids. *Biol. Bull. Wood's Hole* 51: 89-97.
- Harvey, E. N. 1927. Luminous animals. *Scientia* 42: 343-354.
- Harvey, E. N. 1927. Bioluminescence. Rept. of Subcomm. on Chemiluminescence, Bull. Nat. Res. Coun., Wash. No. 59, 50-62.
- Harvey, E. N. 1927. The oxidation-reduction potential of the luciferin oxyluciferin system. *J. gen. Physiol.* 10: 385-393.
- Harvey, E. N. 1927. On the quanta of light produced and the molecules of oxygen utilized during *Cypridina* luminescence. *J. gen. Physiol.* 10: 875-881.
- Harvey, E. N. 1928. Luciferin and luciferase, the luminescent substances of light-giving animals. In *Alexander's Colloid Chemistry*, 2: 395-402.
- Harvey, E. N. 1928. Luciferase. In *Oppenheimer Pincussen, Die Fermente und ihre Wirkungen*, 3: 1400-1413.
- Harvey, E. N. 1928. Studies on the oxidation of luciferin without luciferase and the mechanism of bioluminescence. *J. biol. Chem.* 78: 369-375.
- Harvey, E. N. 1928. The oxygen consumption of luminous bacteria. *J. gen. Physiol.* 11: 469-475.
- Harvey, E. N. 1928. Photosynthesis in absence of oxygen. *Plant Physiol.* 3: 85-89.
- Harvey, E. N. 1928. Stability of luminous substances of luminous animals. *Proc. Soc. exp. Biol.* 26: 133-134.
- Harvey, E. N. 1929. A preliminary study of the reducing intensity of luminous bacteria. *J. gen. Physiol.* 13: 13-20.
- Harvey, E. N. 1929. Phosphorescence. In *Encycl. Brit.* 14th ed.
- Harvey, E. N. 1930. Über Luciferase von leuchtenden Tieren. In *Handb. biol. Arb. Meth.* 4: 827-853.
- Harvey, E. N. 1931. Stimulation by adrenalin of the luminescence of deep sea fish. *Zoologica, N.Y.* 12: 67-69.
- Harvey, E. N. 1931. Chemical aspects of the luminescence of deep-sea shrimp. *Zoologica, N.Y.* 12: 71-74.
- Harvey, E. N. 1931. Photocell analysis of the light of the Cuban elaterid beetle, *Pyrophorus*. *J. gen. Physiol.* 15: 139-145.
- Harvey, E. N. 1932. The evolution of bioluminescence and its relation to cell respiration. *Proc. Amer. phil. Soc.* 71: 135-141.
- Harvey, E. N. 1935. The mechanism and kinetics of bioluminescent reactions. *Cold Spring Harbor Symposia on Quant. Biol.* 3: 261-265.
- Harvey, E. N. 1935. Luciferase, the enzyme concerned in luminescence of living organisms. *Ergebn. Enzymforsch.* 4: 365-379.
- Harvey, E. N. 1939. Bioluminescence. *Trans. Faraday Soc.* 35: 233-235.
- Harvey, E. N. 1939. Deep-sea photography. *Science* 90: 187.
- Harvey, E. N. 1940. *Living Light*. Princeton University Press, 328 pp.
- Harvey, E. N. 1940. Benjamin Franklin's views on the phosphorescence of the sea. *Proc. Amer. phil. Soc.* 83: 341-348.
- Harvey, E. N. 1941. Review of bioluminescence. *Ann. Rev. Biochem.* 10: 531-552.
- Harvey, E. N. 1944. Luminescence. In *Medical Physics*, Chicago, 684-695.
- Harvey, E. N. 1944. The nature of the red and green luminescence of the South American "railroad worm," *Phryxothrix*. *J. cell. comp. Physiol.*, 23: 31-38; *Science* 99: 283-284.
- Harvey, E. N. 1945. Note on the red luminescence and the red pigment of the "railroad worm." *J. cell. comp. Physiol.* 26: 185-187.

- Harvey, E. N. 1947. Bioluminescence. *Encycl. Brit.* 3: 617-619.
- Harvey, E. N. 1948. Introductory remarks: A general survey of bioluminescence. *Ann. N.Y. Acad. Sci.* 49: 329-336.
- Harvey, E. N. 1949. Adenosine triphosphate and the luminescence of the "railroad worm" and other luminous organisms. *Biol. Bull. Wood's Hole* 97: 257-258.
- Harvey, E. N. 1950. Luminescent reactions in the "railroad worm," *Phrixothrix*. *Biol. Bull. Wood's Hole* 99: 360.
- Harvey, E. N., and R. S. Anderson. 1941. Luciferase. In *Meth. Fermentforsch.* 2496-2504.
- Harvey, E. N., and E. R. Baylor. 1948. Deep sea photography. *J. Marine Res.* 7: 10-16.
- Harvey, E. N., and J. E. Deitrick. 1930. The production of antibodies for *Cypridina* luciferase and luciferin in the body of a rabbit. *J. Immunol.* 18: 65-71.
- Harvey, E. N., and R. T. Hall. 1929. Will the adult fire-fly luminesce if its larval organs are entirely removed? *Science* 69: 253-254.
- Harvey, E. N., and I. M. Korr. 1938. Luminescence in absence of oxygen in the ctenophore, *Mnemiopsis leidyi*. *J. cell. comp. Physiol.* 12: 319-323.
- Harvey, E. N., and G. L. Lavin. 1931. Reduction of oxyluciferin by atomic hydrogen. *Science* 74: 150.
- Harvey, E. N., and A. L. Loomis. 1929. The destruction of luminous bacteria by high frequency sound waves. *J. Bact.* 17: 373-376.
- Harvey, E. N., and K. P. Stevens. 1928. The brightness of the light of the West Indian Elaterid Beetle, *Pyrophorus*. *J. gen. Physiol.* 12: 269-272.
- Harvey, E. N., and T. F. Morrison. 1923. The minimum concentration of oxygen for luminescence by luminous bacteria. *J. gen. Physiol.* 6: 13-19.
- Harvey, E. N., and P. A. Snell. 1931. The analysis of bioluminescences of short duration, recorded with photoelectric cell and string galvanometer. *J. gen. Physiol.* 14: 529-545. Also in *Proc. Amer. phil. Soc.* 69: 303-308, 1930.
- Harvey, E. N., and G. W. Taylor. 1934. The oxygen consumption of luminous bacteria in water containing deuterium oxide. *J. cell. comp. Physiol.* 4: 357-362.
- Hasama, B. 1939. Potentialschwankungen am Leuchtorgan des Glühwurms. *Protoplasma* 33: 103-109.
- Hasama, B. 1941. Über die Bioluminescence bei *Chaetopterus varipodatus* Benoit im bioelektrischen sowie histologischen Bild. *Z. wiss. Zool.* 154: 357-372.
- Hasama, B. 1941. Über die Bioluminescenz bei *Watasenia scintillans* im bioelektrische sowie histologischen Bild. *Z. wiss. Zool.* 155: 109-128.
- Hasama, B. 1942. Über die Bioluminescenz bei *Pyrocoelia rufa* im Aktionsstrombild sowie im histologischen Bild. *Ann. zool. Jap.* 21: 59-77.
- Hasama, B. 1942. Über die Bioluminescenz der *Luciola lateralis* im zytologischen Bild sowie im Potentialbild ihres Leuchtorgans. *Cytologia* 12: 366-377.
- Hasama, B. 1942. Über die Bioluminescenz der Larve von *Luciola cruciata* sowie von *Pyrocoelia rufa* im Aktionsstrombild und im histologischen Bild ihres Leuchtorgans. *Cytologia* 12: 378-388.
- Hasama, B. 1942. Zytologische untersuchungen des Leuchtorgans von *Luciola cruciata*. *Cytologia* 12: 389-396.
- Hasama, B. 1942. Zytologische Untersuchungen des Leuchtorgans von zwei tropischen Leuchtkäfern, *Pyrocoelia analis* und *Luciola Gorhami*. *Cytologia* 12: 486-494.

- Hasama, B. 1943. Lebensgeschichte der *Luciola cruciata* und der *Luciola lateralis*. Ann. zool. Jap. 22: 23-47.
- Hasama, B. 1943. Über die Biolumineszenz des *Plecomphorus tilesii* Bergh sowie der *Cavernularia habereri* Moroff im Aktionsstrombild sowie im histologischen Bild. Cytologia 13: 146-154.
- Hasama, B. 1944. Entwicklung des imaginalen Leuchtorgans der *Luciola cruciata* in histologischer sowie bioelektrischer Hinsicht. Cytologia 13: 155-161.
- Hasama, B. 1944. Histologische untersuchungen des Leuchtorgans der *Luciola parvula*. Cytologia 13: 179-185.
- Hassall, A. H. 1841. Supplement to a catalog of Irish zoophytes. Ann. Mag. Nat. Hist. (Ser. 1) 7: 276-287.
- Haswell, W. A. 1882. On the structure and functions of the elytra of the Aphroditean Annelids. Ann. Mag. nat. Hist. 10 (Ser. 5) 238-242; and J. R. micr. Soc. (Ser. 2) 2: 779-780, 1882.
- Haupt, H. 1903. Leuchtende Organismen. Naturw. Wschr. 19 (N.S. 3): 65-71.
- Hayashii, S. 1927. Studies on the luminescent organ of *Watasenia scintillans* (Berry). Fol. Anat. japon. 5: 417-427.
- Hayasi, K., and M. Okuyama. 1929. Bioluminescence, I, II. J. Okayama med. Soc. 41: 185-187, 270-272. In Japanese. Biol. Abstr. 8: 17565, 1934.
- Hayward, R. 1898. A female specimen of *Zarhipis integripennis*. Psyche, Camb., Mass. 8: 179-180.
- Heer, O. 1865. Die Urwelt der Schweiz. Zurich, p. 377.
- Heidt, K. 1936. Über das Leuchten der Collembolen *Onychiurus armatus* Tbg. und *Achorutes muscorium* Templ. Biol. Zbl., 56: 100-109.
- Heinemann, C. 1872. Untersuchungen über die Leuchtorgane der bei Vera-Cruz vorkommenden Leuchtkäfer. Arch. mikr. Anat. 8: 461-471.
- Heinemann, C. 1873. Aschenanalyse von Leuchtorganen mexikanischer Cucujos. Pflüg. Arch. ges. Physiol. 7: 365-367.
- Heinemann, C. 1886. Zur Anatomie und Physiologie der Leuchtorgane mexikanischer Cucujos. Arch. mikr. Anat. 27: 296-382.
- Heinrich, P. 1808. Von der Natur und den Eigenschaften des Lichtes. St. Petersburg, pp. 1-287.
- Heinrich, P. 1815. Ueber phosphoreszenz im Pflanzen und Thierreich. J. Chem. Phys. 13: 266-73.
- Heinrich, P. 1820. Die Phosphoreszenz der Körper, oder die im Dunkeln bemerkbaren Lichtphänomene, etc. (Nürnberg, 1811). III Abhandl. vom Leuchten vegetabilischer, und tierische Substanzen, 313-424.
- Heinrich, P. 1820. Von dem Leuchten vegetabilischer und animalischer Substanzen. J. Chem. Phys. 30: 218-239.
- Heller, J. 1853. Ueber das Leuchten im Pflanzen- und Tierreiche. Arch. physiol. pathol. Chem. Mikr. 6: 44-54, 81-90, 121-137, 161-166, 201-216, 241-251.
- Heller, R. 1917. Biolumineszenz und Stoffwechsel. Int. Z. phys.-chem. Biol. 3: 106-121.
- Henneberg, W. 1899. Leuchtbakterien als Krankheitserreger bei Schwammücken. Zbl. Bakt. (Abt. I) 25: 649-650.
- Henneguy, L. F. 1888. Influence de la lumière sur la phosphorescence des noctiluques. C. R. Soc. Biol. Paris 5 (Ser. 8), 707-708.
- Henneguy, L. F. 1904. Les Insectes. Paris. Organes lumineux, pp. 92-97.

- Hennings, P. 1893. Einige neue und interessante Pilze an der botanische Museum in Berlin. *Hedwigia* 32: 61-64.
- Hennings, P. 1903. Ein stark phosphoreszierender javischen *Agaricus*, *Mycena illuminans* P. Henn. n. sp. *Hedwigia* 42: 309-310.
- Hennings, P. 1904. Ueber leuchtende Hutpilze. *Naturw. Wschr.* 3: 570-571.
- Henry, C. 1896. Utilité en radiographie d'écrans au sulfure de zinc phosphorescent: emission par les vers luisants, de rayons traversant le papier aiguille. *C. R. Acad. Sci. Paris* 123: 400-401.
- Henslow, G. 1879. Frogs and Glow-flies. *Nature, Lond.* 20: 220.
- Hepp, A. 1927. Biologische Beobachtungen (Gross-schmetterling). *Lepid. Rdsch.* 1: 97-98.
- Herdman, W. A. 1904. Phosphorescence phenomenon in the Indian Ocean. *Rep. 73rd meet. Brit. Ass. at Southport, 1903*, p. 695.
- Herdman, W. A. 1913. "Phosphorescence" of Pennatulida. *Nature, Lond.* 91: 582.
- Herdman, W. A. 1913. *Spolia Rumiana. II Funiculina quadrangularis (Pallas), Diazona violacea, Sav, Forbesella tessellata (Forbes); variation in Ascedia; and records of various rare invertebrata.* *J. linnean Soc. (Zool.)* 32: 269-285.
- Herdman, W. A. 1923. Founders of Oceanography and their work. London. Chap. XII, pp. 212-230 on "Phosphorescence" or luminescence in the sea.
- Herfurth, A. H. 1936. Beiträge zur Kenntnis der Bakteriensymbiose der Cephalopoden. *Zeit. Morph. Ökol. Tiere.* 31: 561-607.
- Héricourt, J. 1890. Les microbes lumineux. *Rev. sci., Paris*, 45: 461-467; 46: 490-493, 1891.
- Hermanas, de dos. 1873. Sur les Cucuyos de Cuba. *C. R. Acad. Sci., Paris* 77: 333-334.
- Hernbstadt. 1808. Bemerkungen über das Leuchten organische Körper im Leben und nach der Tode derselben. *Ges. Naturf. Freunde, Mag. neuesten Entdeck. Naturk.* 2: 248-256.
- Hermes. 1887. Demonstration des leuchtenden Nordsee bacillus. *Baumgarten's Jber.* 3: 344.
- Hertwig, R. 1877. Über *Leptodiscus medusoides* eine neue, den Noctilucen verwandte Flagellate. *Jena. Z. Naturw.* 11: 307-323.
- Hess, W. N. 1917. Origin and development of the photogenic organs of *Photuris pennsylvanica* De Geer. *Ent. News* 28: 304-310. Also in *Science*, 47: 143-144 (1918).
- Hess, W. N. 1920. Notes on the biology of some common Lampyridae. *Biol. Bull. Wood's Hole* 38: 39-76.
- Hess, W. N. 1921. Tracheation of the light organs of some common Lampyridae. *Anat. Rec.* 20: 155-161.
- Hess, W. N. 1922. Origin and development of the light organs of *Photuris pennsylvanica*, De Geer. *J. Morph.* 36: 244-277.
- Heyden, C. von. 1861-3. Gliederthiere aus der Braunkohle des Niederrheins, der Wetterau und der Röhn. *Paleaontographica* 10: 62-82.
- Heymans, C. and A. R. Moore. 1923. Action des ions sur la luminescence et les pulsations de *Pelagia noctiluca*. *C. R. Soc. Biol., Paris* 89: 430-432.
- Heymans, C., and A. R. Moore. 1924. Luminescence in *Pelagia Noctiluca*. *J. gen. Physiol.* 6: 273-280.
- Heymans, C., and A. R. Moore. 1925. Note on the excitation and inhibition of luminescence in *Beroë*. *J. gen. Physiol.* 7: 345-348.

- Hickling, C. F. 1925-26. A new type of luminescence in fishes; I, II. J. Mar. biol. Ass. U.K. 13: 914-937; 14: 495-507.
- Hickling, C. F. 1928. The luminescence of the dog-fish, *Spinax niger* Cloquet. Nature, Lond. 121: 280-281.
- Hickling, C. F. 1931. A new type of luminescence in fishes. III. The gland in *Coelorhynchus coelorhynchus*, Risso. J. Mar. biol. Ass. U.K. 17: 853-875.
- Hill, S. E. 1928. The influence of molds on the growth of luminous bacteria in relation to the hydrogen ion concentration, together with the development of a satisfactory culture method. Biol. Bull. Wood's Hole 55: 143-150.
- Hill, S. E. 1928. A simple visual method for demonstrating the diffusion of oxygen through rubber and various other substances. Science 67: 374-376.
- Hill, S. E. 1929. The penetration of luminous bacteria by the ammonium salts of the lower fatty acids. Part I. General outline of the problem, and the effects of strong acids and alkalies. J. gen. Physiol. 12: 863-872.
- Hill, S. E. 1932. The effects of ammonia, of the fatty acids, and of their salts, on the luminescence of *Bacillus fischeri*. J. cell. comp. Physiol. 1: 145-159.
- Hill, S. E., and C. S. Shoup. 1929. Observations on luminous bacteria. J. Bact. 18: 95-99.
- Hincks, T. 1880. A history of the British Marine Polyzoa, London, 2 vols. Introduction p. cxxxv on luminous forms.
- Hirasaka, K. 1922. On a case of discolored sea-water. Annot. zool. jap. 10: 161-164, 1 fig.
- Höllrigl, G. 1908. Lebensgeschichte von *Lamprorhiza splendidula* mit besonderer Berücksichtigung der Leuchtorgane. Ber. naturw.-med. Ver. Innsbruck 31: 167-230.
- Hoffmann, H. 1939. Opisthobranchia. In Bronn's Klassen 3rd vol., 2nd Abt., 3rd. Book, part 1. Müllersche Zelle (Leuchtdrüsenzellen) pp. 411-412.
- Hoffmansegg, J. C. von. 1807. Über das Leuchten von Fulgoren. Mag. Ges. Naturf. Fr. Berlin, 1: 152-155.
- Hofker, J. 1930. Über *Noctiluca scintillans* (Macartney). Arch. Protistenk. 71: 57-78.
- Holder, C. F. 1887. Living Lights. London, 179 pp. Also in Marvels of Animal Life Series, New York, 127 pp. (1892).
- Holder, C. F. 1906. Remarkable phosphorescent animals. Sci. Amer. 94: 135.
- Holt, E. W. L., and L. W. Byrne. 1913. Sixth report on the fishes of the Irish Atlantic Slope. The Families Stomiidae, Sternoptychidae and Salmonidae. Dept. Agric. and Tech. Instruction for Ireland. Sci. Invest. 1912. I.
- Holmrode, I. 1916. Notes on luminosity of Lepidopterous larvae. Nature, Lond. 98: 114.
- Homo, I. 1944. Supplemental knowledge of the neural physiology of *Cavernularia obesa* Valenciennes. Physiol. & Ecolog. Contr. from Otsu Hydrobiol. Expt. Sta., Zool. Lab. Kyoto Univ., No. 9, 13 pp. In Japanese.
- Horne, C. 1869. Note on the phosphorescence of the lobster after death. Zoologist (Ser. 2) 4: 1725-1726.
- Hort, W. P. 1848-1849. An application of the philosophy of various terms of matter, and the laws of motion, to the explanation of the Phosphorescence or Luminosity of animals, plants and gems. N. Orl. Med. Surg. J. 5: 728-743.
- Horsel, C. R. 1942. The effects of bichloride of mercury upon the luminescence and respiration of the luminous bacterium, *Achromobacter fischeri*. J. cell. comp. Physiol. 20: 277-293.

- Hough, W. 1901. The development of illumination. Rep. Smithson. Instn. 1901, 493-500.
- Hoyle, W. E. 1885. Phosorescence. Encyclop. Brit., 9th and 10th ed., 18: 813-814. Also. Trans. Manchr. micr. Soc. 1890, 1-18.
- Hoyle, W. E. 1902. On an Intrapallial Luminous Organ in the Cephalopoda. Verh. 5th int. Zool.-Congr. Berlin, 774.
- Hoyle, W. E. 1902. The luminous organs of *Pterygioteuthis margaritifera*, a Mediterranean Cephalopod. Manchr. Mem. 46: No. 16, 1-14.
- Hoyle, W. E. 1904. "Reports on the Cephalopods" of the "Albatross" expedition in charge of Alex Agassiz (1891, West coast of Mexico). Bull. Mus. comp. Zool. Harv. 43: 1-71.
- Hoyle, W. E. 1908. Presidential address of Section D on Cephalopoda. Rep. 77th Brit. A. A. S., Leicester, 1907, 520-539.
- Hoyle, W. E. 1912. The luminous organs of some Cephalopoda from the Pacific Ocean. I. The eye and luminous organ of *Bathothauma lyromma*. II. Of an undetermined Cranchid. III. The luminous organ of *Onychoteuthis*. Proc. 7th Int. Zool. Congr. 831-835.
- Hsu, H. L. 1937. Ueber den Einfluss des ionisierten Luftbades auf die Bakterien. Sei-i-kwai med. J. 56: 1-11. In German.
- Hubbs, C. L. 1920. The bionomics of *Porichthys notatus*, Girard. Amer. Nat. 54: 380-384.
- Hubbs, C. L., and L. P. Schultz. 1939. A revision of the toadfishes referred to *Porichthys* and related genera. Proc. U.S. nat. Mus. 86: 473-496.
- Hudson, G. V. 1886. A luminous insect-larva in New Zealand. Ent. mon. Mag. 23: 99-100.
- Hudson, G. V. 1887. On New Zealand glow-worms. Trans. Proc. N. Z. Inst. 19: 62-64.
- Hudson, G. V. 1891. The habits and life history of the New Zealand glow-worm. Trans. Proc. N. Z. Inst. 23: 43-47.
- Hudson, G. V. 1926. "The New Zealand Glow-worm," *Boletophila* (Arachnocampa) *luminosa*; Summary of Observations. Ann. Mag. nat. His. (Ser. 9) 17: 228-235 and 18: 667-670.
- Huett, M. 1886. Note sur un Myriapode lumineux trouvé a la Fere (Aisne). C. R. Soc. Biol., Paris (Ser. 8), 3: 523-524.
- Hulme, N. 1800-1801. Experiments and observations on the light which is spontaneously emitted with some degree of permanency from various bodies. Philos. Trans. 1800, 162-187; 1801, 403-426.
- Humboldt, A. von. 1882. Personal Narrative of travels to the equinoctial regions of the new continent during the years 1799-1804. Trans. from French into English by H. M. Williams. London, vol. 1, p. 74.
- Huntsman, A. G. 1948. *Odontosyllis* at Bermuda and lunar periodicity. J. Fish. Res. Board Canada 7: 363-369.
- Huth, W. 1913. Zur Entwicklungsgeschichte der Thalassicollen. Arch. Protistenk. 30: 1-124.
- Huxley, T. H. 1851. Observations on the anatomy and physiology of Salpa and Pyrosoma. Philos. Trans. 1851, 567-593. Also Proc. Linn. Soc. Lond. 1860, 23.
- Huxley, T. H. 1855. On the structure of *Noctiluca miliaris*. Quart. J. micr. Sci. 3: 49-54.
- Hykes, O. V. 1917. Einige Bemerkungen zu dem Aufsatz Isaak's "Ein Fall von

Leuchtfähigkeit bei einem europäischen Grossschmetterling." *Biol. Zbl.*, 37: 106-108.

- Hykes, O. V. 1928. Contribution a la physiologie de la luminescence et de la motilité des Coelentérés. *C. R. Soc. Biol., Paris* 98: 259-261.
- Ihle, J. E. W. 1935. Tunicata Acopa—Caducicordata, Desmomyaria in *Handb. Zool. Berl.* 5 (2nd Half.) 403-532, especially pp. 473-4 and 482.
- Iida, T. T. 1934. Osmotic pressure of *Noctiluca scintillans*. *J. Fac. Sci. Tokyo Univ.* 3: 495-497.
- Illig, G. 1905. Das Leuchten der Gnathophausien. *Zool. Anz.* 28: 662.
- Imai, H. 1942. Studies on the symbiotic luminous bacteria. *Sei-i Kwai med. J.* 61: 454-467. In Japanese.
- Imamura, T. 1904. Ueber ein neues Photobacterium. *J. hyg. chem. Soc. Japan* 1: 48, in Japanese. *Abst. in Zbl. Bakt.* 38: 37 (1906).
- Imms, A. D. 1924. A General Text-book of Entomology, etc. Light producing or photogenic organs of luminous beetles, p. 95, New York.
- Inman, O. L. 1927. A pathogenic luminescent bacterium. *Biol. Bull. Wood's Hole* 53: 197-200.
- Isaak, J. 1916. Ein Fall von Leuchtfähigkeit bei einem europäischen Grossschmetterling. *Biol. Zbl.*, 36: 216-218.
- Ishikawa, C. 1891. Vorläufige Mitteilung über die Conjugations-erscheinungen bei der Noctilucen. *Zool. Anz.* 14: 12-14.
- Ishikawa, C. 1894. Über die Kernteilung bei *Noctiluca miliaris*. *Ber. naturf. Ges. Freiburg i. B.* 8: 54-67.
- Ishikawa, C. 1894. Studies on reproductive elements. II *Noctiluca miliaris* Sur: its division and spore formation. *J. Coll. Sci. Tokyo* 6: 297-334.
- Ishikawa, C. 1899. Further observations on the nuclear division of *Noctiluca*. *J. Coll. Sci. Tokyo* 12: 243-262.
- Ishikawa, C. 1913. Einige Bemerkungen über den leuchtenden Tintenfisch, *Watasenia* nov. gen. (Abraliopsis der Autoren) *scintillans*, Berry, aus Japan. *Zool. Anz.* 43: 162-172.
- Ishikawa, M. 1929. On a new species of luminous squid from the sea of Japan. *Proc. imp. Acad. Japan* 5: 51-54.
- Issatschenko, B. 1903. Quelques expériences avec la lumière bactérienne. *Zbl. Bakt. (Abt. II)*, 10: 497-499.
- Issatschenko, B. 1907. Zur Erforschung des Bakterienlichtes. *Zbl. Bakt. (Abt. II)*, 19: 116-117.
- Issatschenko, B. 1911. Die leuchtende Bakterie aus dem sudlichen Bug. *Bull. Jard. bot. St.-Petersb.*, 11: 44-49.
- Issatschenko, B. 1911. Erforschung des bakterielle Leuchten des Chironomus (Diptera). *Bull. Jard. bot. St.-Petersb.* 11: 31-43, in Russian with German summary.
- Ives, H. E. 1910. Further studies of the firefly. *Phys. Rev.* 31: 637-651. Also in *Elect. World*, N.Y. 56: 864-865 (1910).
- Ives, H. E. 1915. An illuminating engineer's conception of an ideal light. *Trans. Amer. electrochem. Soc.* 27: 419-433.
- Ives, H. E. 1922. The fire-fly as an illuminant. *J. Franklin Inst.* 194: 213-230.
- Ives, H. E., and W. W. Coblentz. 1910. Luminous efficiency of the fire-fly. *Bull. U.S. Bur. Stand.* 6: 321-336. Also in *Proc. 3d Ann. Convent. Illum. Engr. Soc.*, Sept. 30, 1909, N.Y.
- Ives, H. E., and C. W. Jordan. 1913. The intrinsic brilliancy of the glow-worm. *Ltg. J.* p. 47. Also in *Sci. Amer. Supp.* 76: 53.

- Jhering, H. von. 1887. Über eine merkwürdige leuchtende Käferlarve. *Berl. ent. Z.* 31: 11-16.
- Johann, L. 1899. Ueber eigentümliche epitheliale Gebilde (Leuchtorgane) bei *Spinax niger*. *Z. wiss. Zool.* 66: 136-160.
- Johnson, F. H. 1935. A micro-method for determining the utilization of carbohydrates and polyhydric alcohols by microorganisms. *Science* 81: 620-621.
- Johnson, F. H. 1935. Oxidation of carbohydrates and polyhydric alcohols by luminous bacteria. *Proc. Soc. exp. biol.* 32: 1263-1265.
- Johnson, F. H. 1936. The aerobic oxidation of carbohydrates by luminous bacteria, and the inhibition of oxidation by certain sugars. *J. cell. comp. Physiol.* 8: 439-463.
- Johnson, F. H. 1937. Hexose oxidation by luminous bacteria. I. The effect of some natural and synthetic glycosides and related substances. *J. cell. comp. Physiol.* 9: 199-206.
- Johnson, F. H. 1937. An improved Thunberg technique for bacterial oxidations. *Proc. Soc. exp. Biol.* 36: 387-390.
- Johnson, F. H. 1938. Hexose oxidation by luminous bacteria. III. The escape of respiration and luminescence from inhibition by alpha methylglucoside, with a note on urethanes. *J. cell. comp. Physiol.* 12: 281-294.
- Johnson, F. H. 1939. Total luminescence of bacterial suspensions in relation to reactions concerned in luminescence. *Enzymologia* 7: 72-81.
- Johnson, F. H. 1941. Immunological reactions of marine luminous bacteria. *J. Bact.* 41: 67.
- Johnson, F. H. 1942. Mechanism of P-aminobenzoic acid action and the parallel effects of ethyl carbamate (urethane). *Science* 95: 104-105.
- Johnson, F. H. 1947. Bacterial luminescence. *Advanc. Enzymol.* 7: 215-264.
- Johnson, F. H. 1948. Bioluminescence: a reaction rate tool. *Sci. Mon.* 67: 225-235.
- Johnson, F. H., and R. S. Anderson. 1938. Hexose oxidation by luminous bacteria. II. The inhibition of glucose oxidation by alpha methylglucoside. *J. cell. comp. Physiol.* 12: 273-280.
- Johnson, F. H., and E. L. Chambers. 1939. Oxygen consumption and methylene blue reduction in relation to barbital inhibition of bacterial luminescence. *J. cell. comp. Physiol.* 13: 263-267.
- Johnson, F. H., and A. M. Chase. 1942. The sulfonamide and urethane inhibition of Cypridina luminescence in vitro. *J. cell. comp. Physiol.* 19: 151-161.
- Johnson, F. H., and H. Eyring. 1944. The nature of the luciferin-luciferase system. *J. Amer. chem. Soc.* 66: 848.
- Johnson, F. H., and H. Eyring. 1948. Bacteria which make their own light. *J. N.Y. bot. Gdn.* 49: 120-125.
- Johnson, F. H., and H. Eyring. 1948. The fundamental action of pressure, temperature, and drugs on enzymes, as revealed by bacterial luminescence. *Ann. N.Y. Acad. Sci.* 49: 376-396.
- Johnson, F. H., and D. H. Gray. 1949. Nuclei and large bodies of luminous bacteria in relation to salt concentration, osmotic pressure, temperature, and urethane. *J. Bact.* 58: 675-688.
- Johnson, F. H., and E. N. Harvey. 1937. The osmotic and surface properties of marine luminous bacteria. *J. cell. comp. Physiol.* 9: 363-380.
- Johnson, F. H., and E. N. Harvey. 1938. Bacterial luminescence, respiration and

viability in relation to osmotic pressure and specific salts of sea water. *J. cell. comp. Physiol.* 11: 213-232.

Johnson, F. H., and W. D. Lynn. 1940. The flash of luminescence following anaerobiosis of fungus mycelium. *Anat. Rec.* 78 (supp.), 65.

Johnson, F. H., and K. Moore. 1941. Sulphonamide inhibition of bacterial luminescence. *Proc. Soc. exp. Biol.* 48: 323-325.

Johnson, F. H., and L. Schneyer. 1944. The quinine inhibition of bacterial luminescence. *Amer. J. trop. Med.* 24: 163-175.

Johnson, F. H., and K. L. van Schouwenburg. 1939. Decomposition of hydrogen peroxide by catalase. *Nature, Lond.* 144: 634-635.

Johnson, F. H., and I. V. Shunk. 1936. An interesting new species of luminous bacteria. *J. Bact.* 31: 585-592.

Johnson, F. H., D. Brown, and D. Marsland. 1942. A basic mechanism in the biological effects of temperature, pressure and narcotics. *Science* 95: 200-203.

Johnson, F. H., D. E. S. Brown, and D. A. Marsland. 1942. Pressure reversal of the action of certain narcotics. *J. cell. comp. Physiol.* 20: 269-276.

Johnson, F. H., C. M. Carver, and W. K. Harryman. 1942. Luminous bacterial auxanograms in relation to heavy metals and narcotics, self photographed in color. *J. Bact.* 44: 703-714.

Johnson, F. H., H. Eyring, and W. Kearns. 1943. A quantitative theory of synergism and antagonism among diverse inhibitors, with special reference to sulphanilamide and urethane. *Arch. Biochem.* 3: 1-31.

Johnson, F. H., H. Eyring, and R. W. Williams. 1942. The nature of enzyme inhibitions in bacterial luminescence: sulfanilamide, urethane, temperature and pressure. *J. cell. comp. Physiol.* 20: 247-268.

Johnson, F. H., D. Rexford, and E. N. Harvey. 1949. The hypothetical structure of luciferin. *J. cell. comp. Physiol.* 33: 133-136.

Johnson, F. H., K. L. van Schouwenburg, and A. van der Burg. 1939. The flash of luminescence following anaerobiosis of luminous bacteria. *Enzymologia* 7: 195-224.

Johnson, F. H., N. Zworykin, and G. Warren. 1943. A study of luminous bacterial cells and cytolysates with the electron microscope. *J. Bact.* 46: 167-184.

Johnson, F. H., E. A. Flagler, R. Simpson, and K. McGeer. 1951. The inhibition of bacterial luminescence by a homologous series of carbamates. *J. cell. comp. Physiol.* 37: 1-14.

Johnson, F. H., H. Eyring, R. Steblay, H. Chaplin, C. Huber, and G. Gerhardt. 1945. The nature and control of reactions in bioluminescence. With special reference to the mechanism of reversible and irreversible inhibitions by hydrogen and hydroxyl ions, temperature, pressure, alcohol, urethane, and sulphanilamide in bacteria. *J. gen. Physiol.* 28: 463-537.

Johnson, G. 1847. *A History of the British Zoophytes*. 2nd ed. 2 vols. Vol. 1, pp. 150-155 on phosphorescence. 1st ed. 1838.

Jones, H., G. Rake, and D. M. Hamre. 1943. Studies on *Aspergillus flavis*. I. Biological properties of crude and purified aspergillic acid. *J. Bact.* 45: 461-469.

Jordan, D. S. 1926. Cold lights of the sea: fishes which inhabit great depths carry their own lanterns. *Sci. Amer.* 82: 247-248; and *Wiss. u. Fortschr.* (1927), 310-313.

Joseph, G. 1854. Beobachtungen über das leuchten der Johanniskäfer. *Z. Ent.* 8: 1-12.

- Josserand, M. 1937. Un mot sur la luminescence de "Clitocybe olearia." Bull. mens. Soc. linn. Lyon 6: 46.
- Joubin, L. 1893. Note sur une adaptation particuliere de certains chromatophores chez un Cephalopode. L'oeil thermoscopique de *Chiroteuthis Bomplandi* Verany. Bull. Soc. zool. Fr. 18: 147-151.
- Joubin, L. 1893. Recherches sur l'appareil lumineux d'un Cephalopode; *Histioteuthis Ruppellii*, Verany. Rennes, 32 pp., and Bull. Soc. sci. méd. Ouest 2: 49-78. Also C. R. Soc. Biol., Paris 44: 142-6.
- Joubin, L. 1894. Nouvelle recherches sur l'appareil lumineuse des Cephalopodes du genre *Histioteuthis*. Bull. Soc. sci. méd. Ouest 3, July 6.
- Joubin, L. 1895. Note sur les appareils photogenes cutanes de deux cephalopodes: *Histiopsis atlantica* Hoyle et *Abralia oweni* (Verany) Hoyle. Mem. Soc. zool. Fr. 8: 212-228.
- Joubin, L. 1895-1924. Les cephalopodes. Result. Camp. sci. Monaco, 1895, Fasc. 9; 1900, Fasc. 17; 1920, Fasc. 54; 1924, Fasc. 57.
- Joubin, L. 1905. Note sur les organes lumineux de deux Céphalopodes. Bull. Soc. zool. Fr. 30: 64-69.
- Joubin, L. 1905. Note sur les organes photogènes de l'oeil de *Leachia cyclura*. Bull. Mus. oceanogr. Monaco, No. 33, 13 pp.
- Joubin, L. 1905. Cours d'Océanographie, 185 pp. Leçon III and IV Les animaux lumineux. Bull. Mus. oceanogr. Monaco, No. 45, 69-111.
- Jourdan, Et. 1885. Structure des élytres de quelques Polynœs. Zool. Anz. 8: 128-134.
- Joya, M. 1912. The lore and legend of Japanese fireflies. Strand Mag., 44: (July) 72-77.
- Joyeux-Laffuie, J. 1890. Étude monographique du Chétoptère (*Chaetopterus variopedatus*, Rénier). Arch. Zool. exp. gén. (Ser. 2) 8: 245-360.
- Julin, C. 1909. Les embryons de *Pyrosoma* sont phosphorescents; les cellules du testa (*Calymmocytes* de Salensky) constituent les organes lumineux du cyathozoïde. C. R. Soc. Biol., Paris 66: 80-82.
- Julin, C. 1912. Recherches sur le développement embryonnaire de *Pyrosoma giganteum* Les. I. Aperçu général de l'embryogenèse. Les cellules du testa et le développement des organes lumineux. Zool. Jb. Suppl. 15 (part 2) 775-863.
- Julin, C. 1912. Les caractères histologiques spécifiques des "cellules lumineuses" de *Pyrosoma giganteum* et de *Cyclosalpa pinnata*. C. R. Acad. Sci., Paris 155: 525-527.
- Julin, C. 1913. The specific histological characters of the "luminous cells" of *Pyrosoma giganteum* and of *Cyclosalpa pinnata*. Rep. 82d meet. Brit. Ass. Dundee, 1912, 492-493.
- Kaiser, M. W. 1884. Ueber das Leuchten von *Lampyrus splendidula*, L. Ann. Akad. Wiss. Wien No. 17: 133-134. Rev. in Ann. Mag. Nat. Hist. 14 (Ser. 5): 372.
- Kajiyama, E. 1912-1913. Study on Ostracoda of Misaki (1-3). Zool. Mag. Tokyo 24 (287): 488-492. 24 (289): 609-619. 25 (291): 1-16.
- Kanda, S. 1920. Physico-chemical studies on bioluminescence. I. On the luciferine and luciferase of *Cypridina Hilgendorffii*. Amer. J. Physiol. 50: 544-560.
- Kanda, S. 1920. Physico-chemical studies on bioluminescence. II. The production of light by *Cypridina Hilgendorffii* is not an oxidation. Amer. J. Physiol. 50: 561-573.

- Kanda, S. 1920. Physico-chemical studies on bioluminescence. III. The production of light by *Luciola viticollis* is an oxidation. *Amer. J. Physiol.* 53: 137-149.
- Kanda, S. 1921. Physico-chemical studies on bioluminescence. IV. The physical and chemical nature of the luciferase of *Cypridina Hilgendorffii*. *Amer. J. Physiol.* 55: 1-12.
- Kanda, S. 1924. Physico-chemical studies on bioluminescence. V. The physical and chemical nature of the luciferine of *Cypridina Hilgendorffii*. *Amer. J. Physiol.* 68: 435-444.
- Kanda, S. 1928. Physico-chemical studies on bioluminescence. VI. The mechanism of luminescence in the *Cypridina* luciferin and luciferase suggested. *Sci. Pap. Inst. Phys. Chem. Res. Tokyo* 9: 265-269.
- Kanda, S. 1929. Physico-chemical studies on bioluminescence. VII. The solubility of *Cypridina* luciferin in organic solvents. *Sci. Pap. Inst. Phys. Chem. Res. Tokyo*, 10: 91-98.
- Kanda, S. 1930. The chemical nature of *Cypridina* luciferin. *Science* 71: 444. Also in *Sci. Pap. Inst. Phys. Chem. Res. Tokyo*, 13: 246-247.
- Kanda, S. 1932. Crystalline luciferin. *Supp. to Sci. Pap. Inst. Phys. Chem. Res. Tokyo*, 18: 1.
- Kanda, S. 1938. The luminescence of *Pontodrilus matsushimensis*. *Rigakukai.* 36: (3) 1-7.
- Kanda, S. 1939. The luminescence of a Nemertean, *Emplectonema Kandai*. *Kato. Biol. Bull. Wood's Hole* 77: 166-173.
- Karrer, E. 1918. The efficiency of light-production in organisms. *J. Franklin Inst.* 185: 775-783.
- Kastle, J. H., and F. A. McDermott. 1910. Some observations on the production of light by the firefly. *Amer. J. Physiol.* 27: 122-151.
- Kato, K. 1939. A new luminous species of the Nemertea, *Emplectonema kandai* sp. nov. *Jap. J. Zool.* 8: 251-254. In English.
- Kato, K. 1947. Luminous Ophiuroidea. *Zool. Mag. Tokyo* 57: 10. In Japanese.
- Kato, K. 1947. A new type of luminous organ of fishes. *Zool. Mag. Tokyo*, 57: 195-198. In Japanese.
- Kato, K. 1949. Luminous organ of *Kaloplocamus ramosum*. *Zool. Mag. Tokyo*, 58: 163-164. In Japanese.
- Katz, O. 1887. Preliminary remarks on phosphorescent bacteria from sea water. *Proc. Linn. Soc. N. S. W.* 2 (Ser. 2): 331-336.
- Katz, O. 1891. Zur Kenntniss der Leuchtbakterien. *Zbl. Bakt.*, 9: 157-163; 199-204; 229-234; 258-264; 311-316; 343-350.
- Kauzmann, W. J., A. M. Chase, and E. H. Brigham. 1949. Studies on cell enzyme systems. III. Effect of temperature on the constants in the Michaelis-Menten relation for the luciferin-luciferase system. *Arch. Biochem.* 24: 281-288.
- Kavanagh, F. 1947. Antiluminescent activity of antibacterial substances. *Bull. Torrey bot. Cl.* 74: 414-425.
- Kawamura, S. 1915. Studies on the luminous fungus, *Pleurotus japonicus*, sp. nov. *J. Coll. Sci. Tokyo* 35: art. 3, 29 pp.
- Kawanaka, T., and H. Matsubara. 1941. Study of lanternflies, I, Chemical components of *Luciola cruciata* MOTSCHULSKY. *Sci. and Ind. Tokyo*, 16: 441-444, and 17: 480-486. In Japanese. Review in *Chem. Abstr.* 36: 169.
- Keenan, G. L. 1926. Substances which affect photographic plates in the dark. *Chem. Rev.* 3: 95-111.

- Kemp, S. 1910. Notes on the photophores of decapod crustacea. *Proc. zool. Soc. Lond.*, 1910 (pt. II): 639-651.
- Kemp, S. 1925. Notes on crustacea decapoda in the Indian Museum. XVII. On various Caridea. *Rec. Indian Mus.*, 27: 249-343.
- Kent, W. S. 1873. Phosphorescence in fish. *Nature, Lond.* 7: 47-48.
- Kent, W. S. 1880-1881. A manual of infusoria. London, vol. I, pp. 396-401 on Noctilucidae.
- Kenyon, F. C. 1893. A preliminary list of the myriapoda of Nebraska, with descriptions of new species. *Publ. Neb. Acad. Sci.* 3: 14-18.
- Kershaw, J. C. W., and G. W. Kirkaldy. 1910. A memoir on the anatomy and life-history of the homopterous insect, *Pyrops candelaria* (or "Candle-fly"). *Zool. Jb. (Abt.)* 29: 105-124.
- Khvorostansky, C. 1892. Sur la lumination des animaux de la mer Blanche. *Congr. Intern. Zool., Moscou*, 1892, 2 sess., pt. 4, 185-186.
- Kiernik, E. 1908. Über einige bisher unbekannte leuchtende Tiere. *Zool. Anz.* 33: 376-380.
- King, G. S. 1949. Production of red tide in the laboratory. *Proc. Gulf Caribb. Fish. Inst.*, 2nd session. Nov. 1949.
- King, V. O. 1878. Phosphorescent insects; their metamorphoses. *Amer. Nat.* 12: 354-358.
- King, V. O. 1880. Life history of *Pleotomus pallens*. *Psyche, Camb., Mass.* 3: 51-53.
- King-Li-Pin, Tschang-Si, Tai-lee & Lin-yu-su. 1935. Etude de la variation corporelle et de l'action des cations sur la photogénèse de *Cavernularia haberi*, Moroff. *Contr. Inst. Physiol. Nat. Acad. Peiping* 3: 87-94.
- Kirby, W., and W. Spence. 1817. An Introduction to Entomology. On luminous insects, 2, Letter XXV, pp. 408-429.
- Kirkaldy, G. W. 1901. Notes on some Rhynchota collected chiefly in China and Japan by Mr. T. B. Fletcher R. N., F. E. S. *Entomologist* 1901: 50.
- Kisch, A. M. 1909. A biological study of *Noctiluca miliaris*, Suriray. *Amer. Midl. Nat.* 1: 8-16.
- Kishitani, T. 1928. On the luminous organs of *Watasenia scintillans*. *Annot. zool. Jap.* 11: 353-361.
- Kishitani, T. 1928. Drei neue arten von Leuchtbakterien. *Proc. imp. Acad. Japan*, 4: 69-75.
- Kishitani, T. 1928. Über das Leuchtorgan von *Euprymna morsei* Verrill und die symbiontische Leuchtbakterien. *Proc. imp. Acad. Japan*, 4: 306-309.
- Kishitani, T. 1928. Preliminary report on the luminous symbiosis in *Sepiola birostrata*, Lasaki. *Proc. imp. Acad. Japan*, 4: 393-396.
- Kishitani, T. 1928. L'étude de l'organe photogene du *Loligo edulis*, Hayle (Notes preliminaire). *Proc. imp. Acad. Japan*, 4: 609-612.
- Kishitani, T. 1930. Studien über die Leuchtsymbiose in *Physiculus japonicus* HILGENDORF, mit der Beilage der zwei neuen Arten der Leuchtbakterien. *Sci. Rep. Tohoku Univ.* 5: 801-823.
- Kishitani, T. 1932. Studien über Leuchtsymbiose von japanischen Sepien. *Folia anat. Japon*, 10: 315-418 and *Stud. Tokugawa Inst.* 2: 315-418.
- Kishitani, T. 1933. Zur morphologie und Biologie einer Leuchtbakterienart. *J. Sci. Hiroshima Univ.*, B, 1: 183-196.
- Klem, G. 1928. Die Lichtentwicklung bei Pflanzen. *Handb. norm. u. pathol. Physiol.* 8 (2), 1057-1071.

- Kluyver, A. J., G. J. M. Van der Kerk, and A. Van der Burg. 1942. The effect of light on light emission by luminous bacteria. *Proc. Ned. Akad. Wetensc.* I. 45: 886-895; II. 45: 962-967.
- Knab, F. 1895. Ant Nests. *Ent. News* 6: 15-16.
- Knab, F. 1905. Observations on lampyridae. *Canad. Ent.* 37: 238-239.
- Knab, F. 1909. Luminous termite Hills. *Science* 30: 574-575.
- Knauer, F. 1910. Neues über unsere Leuchtkäfer. *Prometheus* 21: 393-397.
- Knaus, W. 1907. Phengodes Illiger—a note on luminous females and larvae. *Ent. News* 18: 318-319.
- Knapp, C. T. 1939. On the path of the firefly while periodically flashing. *Science* 89: 386-387.
- Knop, J. 1926. Bakterien und Bakteroiden bei Oligochäten. *Z. Morph. Ökol. Tiere* 6: 588-624.
- Kobayashi, Y. 1937. Several luminous Mycomycetes from the Bonin Islands. *Bull. biogeogr. Soc. Japan* 7: 1-7.
- Kobayashi, Y. 1949. A luminous fungus newly found in Japan. *Bull. Nat. Sci. Mus.* No. 26: 13-19. In Japanese with English summary.
- Koch, A. 1927. Studien an leuchtenden Tieren. I. Das Leuchten der Myriapoden. *Z. Morph. Oekol. Tiere* 8: 241-270.
- Koefoed, E. 1944. Pediculati. Results "Michael Sars" N. Atlantic deep sea exped., 1910, 17 pp.
- Kölliker, A. von. 1853. Eigenthümliche Hautorgane u. Wirbel von Chauliodus. *Z. wiss. Zool.* 4: 366-367.
- Kölliker, A. von. 1857. Ueber die Leuchtorgane von Lampyris; Eine vorläufige Mittheilung. *Mber. preuss. Akad. Wiss.* 392; also in *Verh. Würzburg Phys. Med. Ges.* 8: 217-224; and *Quart. J. micr. Sci.* (1858) 6: 166-173.
- Koernicke, M. 1904. Die Wirkung der Radiumstrahlen auf die Keimung und das Wachstum. *Ber. deutsch. chem. Ges.* 22: 155-166.
- Kofoed, C. A. 1905. Craspedotella, a new genus of the Cystoflagellata an example of Convergence. *Bull. Mus. comp. Zool. Harv.* 46: 163-166.
- Kofoed, C. A. 1911. Dinoflagellata of the San Diego Region. IV. The genus Gonyaulax, with notes on its skeletal morphology and a discussion of its generic and specific characters. *Univ. Calif. Publ. Zool.* 8: 188-269.
- Kofoed, C. A. 1920. A new morphological interpretation of the structure of *Noctiluca*, and its bearing on the status of Cysto-flagellata (Haeckel). *Univ. Calif. Publ. Zool.* 19: 317-334.
- Kofoed, C. A., and O. Swezy. 1921. The free-living unarmoured dinoflagellata. *Mem. Univ. Calif.* 5: 1-562.
- Kolbe, H. J. 1887. Beobachtungen über Termiten und Leucht-Käfer (Lampyris) im Caplande; nach briefl. Mittheilungen des H. Dr. Franz Bachmann. *Ent. Nachr.* 13: 70-74; see also 36.
- Komarek, J. 1934. Luminescence of carpathian worms and its causes. *Bull. int. Acad. Prague* 44: 1-10.
- Komarek, J., and K. Wenig. 1938. Die Eigenschaften des Leuchtens der Eisenia submontana Vejd. (Vermes-Olig.) und die Bedeutung der Biolumineszenz im Tierreich. *Vestn. čsl. Spolec. nauk.* (article 12), 1-12.
- Korr, I. M. 1935. The relation between cell integrity and bacterial luminescence. *Biol. Bull. Wood's Hole* 68: 347-354.
- Korr, I. M. 1935. An electrometric study of the reducing intensity of luminous

- bacteria in the presence of agents affecting oxidations. *J. cell. comp. Physiol.* 6: 181-216.
- Korr, I. M. 1936. The luciferin-oxyluciferin system. *J. Amer. chem. Soc.* 58: 1060-1061.
- Kortum, B. K. 1800. Ueber die Phosphorescenz vegetabilischer, in Fäulniss gehender, Körper. *Voigt's Mag. Neuest. Zust. Naturk.* 2: 67-70.
- Kostka, G. 1928. Lebende Bakterien als Sauerstoff-indikatoren. *Mikrokosmos* 22: 6-11, 27-30.
- Krause, E. 1881. Die "augenanlichen" Organe der fische nach den Untersuchung von Dr. Ussow, Prof. Leydig u. A. *Kosmos, Stuttgart*, 9: 433-438.
- Kreezer, G. L., and E. H. Kreezer. 1947. The form of the light-response of luminous bacteria to a sudden increase in temperature and its analysis as a transient. *J. cell. and comp. Physiol.* 30: 173-202.
- Krekel, A. 1920. Die Leuchtorgane von *Chaetopterus variopedatus*, Clap. *Z. wiss. Zool.* 118: 480-509.
- Krogh, A. 1939. Osmotic Regulation in aquatic animals. Cambridge Univ. Press, 242 pp. See p. 11.
- Krohn, A. 1852. Notiz ueber der *Noctiluca miliaris*. *Surir. (Mammaria scintillans, Ehr.) Wiegmann's Arch. Naturgesch.* 18: (part 1), 76-81.
- Krukenberg, C. F. W. 1887. Neue Tatsachen für eine vergleichende Physiologie der Phosphorescenzercheinungen bei Thieren und bei Pflanzen; *Vergl. Physiol. Studien, Experimentelle Untersuchungen II Reihe, 4 Abt., Heidelberg*, pp. 77-141. Review in *Zbl. Physiol.* (1887) 1: 689-691.
- Kuhn, J. 1927. Príspevek, K. biológii, pathogenite a serologusviticich bakterii. In *Slovak. Biol. Spesy. Vysoke Skoly Zverolekarske, Brno.* 6: 137-143.
- Kuhnt, P. 1907. Das Leuchten der Lampyriden. *Ent. Wschr.* 24: 3-4.
- Kusnezoff, I. D. 1890. On the luminescence of diptera. (In Russian.) *Westn. Estestwasnanijo St. Petersburg No. 4*: 167-171.
- Kutscher, F. 1893, 95. Ein Beitrag zur Kenntniss der den Chloreaivirionen ähnlichen Wasserbakterien. *Dtsch. med. Wschr.* 15: 1301-1303; also in *Zbl. Bakt.* 15: 44-46 (1894), and 18: 424, 1895.
- Kutscher, F. 1897. Zur Physiologie der Phosphorescence. *Hoppe-Seyl. Z.* 23: 109-114.
- Kutschera, F. 1909. Die Leuchtorgane von *Acholoe astericola*. *Z. wiss. Zool.* 92: 75-102.
- Laboulbene, A. 1882. Note sur la ver luisant (*Lampyris noct. L.*). *Ann. Soc. ent. Fr. (Ser. 6)* 2: 316.
- Lacordaire, T. 1838. Introduction a l'entomologie. 2 vols. Paris. Vol. 2, pp. 140-150 (Suites a Buffon, Insectes) on "Matière phosphorique" in Chap. X on Nutrition.
- Lafar, F. 1910. Technical Mycology. Trans. from German by C. T. C. Salter. London. 2 vols. Vol. 1, Chap. XV Photogenic Bacteria, pp. 123-126.
- Lagerheim, G. von. 1889. Um novo *Polyporus phosphorescente* de Angola e observações sobre a explicação biologia dos coguemelos luminosos. *Bol. Soc. broteriana* 7: 179.
- Landsborough, D. 1842. On the phosphorescence of zoophytes. *Ann. Mag. nat. Hist. (Ser. 1)* 8: 257-260.
- Langley, E. P., and F. E. Fowle, Jr. 1908. The cheapest form of light. *Ann. Astrophys. Obs. Smithson. Instn.* 2: 5.
- Langley, S. P., and F. W. Very. 1890. On the cheapest form of light, from studies

- at the Allegheny Observatory. Amer. J. Sci. (Ser. 3) 40: 97-113; also in Smithson. misc. Coll., 41: (1901).
- Lankester, E. R. 1868. Preliminary notice of some observations with the spectro-scope on animal substances. J. Anat. Lond. 2: 114-116.
- Lassar, O. 1880. Die Mikrokokken der Phosphoreszenz. Pflüg. Arch. ges. Physiol., 21: 104-108.
- Latreille, P. A. 1829. Sur la phosphorescence de la tache ocellée qui existe sur chacune des elytres d'un Bupreste de l'Inde, Buprestis ocellata, Fabr. Cuvier's Le Regne Animal, Paris, 2nd ed., 4: 447.
- Leavitt, B. B. 1935. A quantitative study of the vertical distribution of the larger zoöplankton in deep water. Biol. Bull. Wood's Hole 68: 115-130, 74: 376-394 (1938).
- Leavitt, B. B. 1938. The quantitative vertical distribution of macrozooplankton in the Atlantic Ocean basin. Biol. Bull. Wood's Hole 74: 376-394.
- Lebenbaum, M. 1930. L'influence des ions sur la luminescence bacterienne. Acta Soc. Bot. Polon. 7: 583-597. Polish with French summary.
- LeConte, J. L. 1880. On lightning-bugs. Summarized in Proc. Amer. Ass. Adv. Sci. 650-659; also Canad. Ent. 12: 174-184.
- Lefebure, M. 1834. No title (on Fulgora). Ann. Soc. ent. Fr. 3: Bull. 63.
- Lehmann, Dr. 1862. Zur Lampyrispreisfrage. Nova Acta Leop. Carol., 30: 113-114.
- Lehmann, K. B. 1889. Ueber die Biologie des Bacterium phosphorescens Fischer. S.B. phys.-med. Ges. Würzburg (1890), 42-44; also in Zbl. Bakt. 5: 785-791.
- Lehmann, K. B., and Sano. 1908. Über das Vorkommen von Oxydationsfermenten bei Bakterien und höheren Pflanzen. Arch. Hyg. Berlin 67: 99-113.
- Lendenfeld, R. von. 1887. Report on the structure of the phosphorescent organs of fishes. Challenger Rep. 22: Appendix B, pp. 277-329.
- Lendenfeld, R. von. 1887. Die Leuchtorgane der Fische. Biol. Zbl. 7: 609-621.
- Lendenfeld, R. von. 1887. Report on the structure of the phosphorescent organs of fishes. Challenger Rep. Zool. 22: 277-329.
- Lendenfeld, R. von. 1905. The radiating organs of the deep sea fishes. Mem. Harv. Mus. comp. Zool. 30: 169-213.
- Lereboullet, A. 1864. Remarques sur les observations de M. Leuckart relatives a des organes oculiformes chez quelques poissons. Ann. Sci. nat. (Zool.) (Ser. 5) 2: 355.
- Lesueur, M. 1815. Mémoire sur l'organisation des Pyrosomes et sur la place qu'ils semblent devoir occuper dans une classification naturelle. Bull. Sci. Soc. philom. Paris 4: 70-74; see also 3 (1813): 281-285.
- Leuckart, R. 1865. Ueber mutmassliche Nebenaugen bei einem Fische, Chauiodus sloani. Ber. dtsh. Naturf. Aerz. 39: 153-155.
- Leydig, F. 1857. Lehrbuch der vergleichende Histologie der Menschen und der Tiere. Frankfurt a. M., pp. 342-344, 551 pp.
- Leydig, F. 1879. Ueber die Nebenaugen von Chauiodus Sloanii. Arch. Anat. Physiol. Lpz. (Anat. Abt.) 365-382.
- Leydig, F. 1881. Die augenähnlichen Organe der Fische. Bonn, 100 pp.
- Leydig, F. 1903. Bemerkung zu den "Leuchtorganen" der Selachier. Anat. Anz. 22: 297-301.
- Landmann, C. 1863. Anatomische Untersuchungen über die Structur des Leuchtorgans von Lampyris splendidula. Bull. Soc. Imp. Nat. Moscow, 36: (Pt. 2): 437-456.

- Link, H. F. 1808. Ueber die Chemischen Eigenschaften des Licht. St. Petersburg 92 pp.
- Linsbauer, K. 1917. Selbstleuchtende Regenwürmer. *Umschau* 21: 67-69.
- Lloyd, R. E. 1907. Notes on phosphorescence in marine animals, with a description of a new polychaete worm. *Rec. Indian Mus.* 1: 257-261.
- Lloyd-Bozward, J. 1897. A colony of highly phosphorescent earthworms. *Nature*, Lond. 56: 544.
- Lo Bianco, S. 1909. Notizie biologiche. *Mitt. zool. Sta. Neapel* 19: 513-761.
- Lode, A. 1904. Versuche, die optische Lichtintensität bei Leuchtbakterien zu bestimmen. *Zbl. Bakt. (Abt. 1)* 35: 524-527.
- Lode, A. 1908. Experimente mit Leuchtbakterien. *Ber. naturw. med. Ver. Innsbruck* 31: 23-24; Abstract in *Zbl. Bakt. (1908) (Abt. 2)* 22: 421.
- Loew, O. 1901. Catalase, a new enzyme of general occurrence with special reference to the tobacco plant. *Rep.* 68, U.S. Dep. Agric. Vegetable Physiol. Pathol. Div.: 35-36.
- Lohmann, H. 1899. Das Gehäuse der Appendicularien, sein Bau, seine Function und seine Entstehung. *Schr. naturw. Ver. Schl.-Holst.* 2: 345-407.
- Lohmann, H. 1933. Tunicata Appendicularie in Kükenthal-Krumbach *Handb. Zool. Berlin* 5 (2nd Half), 1-202, especially, 143-144 and 51, 105, 106.
- Lowe, R. T. 1842. Description of a new dorsibranchiate gasteropod, discovered at Madeira. *Proc. zool. Soc. Lond.* 51-53.
- Lucas, H. 1887. Description d'une larve de Lampyridae considérée comme devant appartenir au sexe femelle, dont l'insecte parfait est encore inconnu. *Ann. Soc. ent. Fr. (Ser. 6)* 7: Bull. xxxv-xxxvi.
- Lucas, H. 1888. Phosphorescence des eufs du *Lampyris noctiluca*. *Ann. Soc. ent. Fr. (Ser. 6)* 8: Bull. cxxxiii-cxxxiv.
- Ludwig, F. 1874. Über die phosphorescence der Pilze und des Holzes. *Inaug. Dissert. Göttingen*, 30 pp.
- Ludwig, F. 1882. Pilzwirkungen. *Programm.-Städt. Gymnas. Greiz*.
- Ludwig, F. 1882. Über einem neuen einheimischen phosphoreszierenden Pilz. *Agaricus (Collybia) tuberosus* Bull. *Bot. Zbl.* 12: 104-106.
- Ludwig, F. 1884. Über die Spektroskopische untersuchung photogener Pilze. *Zeit. wiss. Mikr.* 1: 181-190.
- Ludwig, F. 1884. *Micrococcus Pflugeri*, ein neuer photogener Pilz. *Hedwigia*. 23: 33-37.
- Ludwig, F. 1887. Die bisherigen untersuchungen ueber photogene Bakterien. *Zbl. Bakt.*, 2 (Abt. 1): 372-376, 401-406.
- Ludwig, F. 1890. Über einige neue Pilze aus Australien. *Bot. Zbl.*, 43: 5-9.
- Ludwig, F. 1891. Über die Phosphorescence von *Grylotalpa vulgaris*. *Zbl. Bakt.*, 9: 561-562.
- Ludwig, F. 1892. *Lehrbuch der niederen Kryptogamen*. Stuttgart.
- Ludwig, F. 1898. Leuchten unsere Susswasserperideneen? *Bot. Zbl.* 76: 295-300.
- Ludwig, F. 1901. Phosphoreszierende Tausendfüßler und die Lichtfäule des Holzes. *Zbl. Bakt. (Abt. 2)* 7: 270-274.
- Ludwig, F. 1902. Leuchtende Ameisen. *Ill. Ztg.* 118: 562.
- Ludwig, F. 1904. Phosphoreszierende Collembolen. *Prometheus* 16: 103-107.
- Ludwig, W. 1928. Permeabilität und Wasserwechsel bei *Noctiluca miliaris* Suriray. *Zool. Anz.* 76: 273-285.
- Lüders, L. 1909. *Gigantocypris agassizi* (Müller). *Z. wiss. Zool.*, 92: 103-148.

- Lund, E. J. 1911. On light reactions in certain luminous organisms. *Johns Hopk. Univ. Circ.* 30: 10-13.
- Lund, E. J. 1911. On the structure, physiology and use of photogenic organs, with special reference to the Lampyridae. *J. exp. Zool.* 11: 415-467.
- Lund, E. J., and G. A. Logan. 1925. The relation of stability of protoplasmic films in *Noctiluca* to the duration and intensity of an applied electric potential. *J. gen. Physiol.* 7: 461-473.
- Lutz, L. 1931. Sur la luminescence du mycelium de l'*Armillaria mellea*, Vahl, Action des anti-oxygènes. *Travaux Cryptogramique ded. a L. Mangin Paris. Mus. Nat. d'Hist. Nat. Paris*, 1-4.
- Lyon, E. P. 1923. Effects of electricity on *Noctiluca*. *Proc. Soc. exp. Biol., N.Y.* 20: 284-285.
- Macaire, J. 1821. Mémoire sur la phosphorescence des Lampyres. *J. Phys.* 93: 46-56. Also *Ann. Chim. (Phys.)* 17: 151-167.
- Macaire, J. 1822. On the phosphorescence of luminous insects. *Quart. J. Sci. Lit. Arts* 12: 181-182. Also *Ann. Phys., Lpz.* 70: 265-280.
- Macartney, J. 1810. Observations upon luminous animals. *Philos. Trans.* 100: 258-293.
- MacCulloch, J. 1821. Remarks on marine luminous animals. *Quart. J. Sci. Lit. Arts* 11: 248-260. Also *Edinburgh Phil. J.* 5: 388-9.
- Macé, M. 1886. Sur la phosphorescence des Géophiles. *C. R. Acad. Sci., Paris*, 103: 1273-1274.
- Macé, M. 1887. Les glandes préanales et la phosphorescence des géophiles. *Compt. rend. Soc. Biol. Paris (Ser. 8)* 4: 37-39.
- Macfadyen, A. 1900. On the influence of the temperature of liquid air on bacteria. *Proc. roy. Soc.* 66: 180-182, 339-340.
- Macfadyen, A. 1902. On the influence of the prolonged action of liquid air on micro-organisms and the effect of mechanical trituration at the temperatures of liquid air on photogenic bacteria. *Proc. roy. Soc.* 71: 76-77; also in *Chem. News* 88: 193 and *Nature, Lond.* 68: 608-9, 1903.
- Macfadyen, A., and S. Roland. 1900. Influence of the temperature of liquid hydrogen on bacteria. *Proc. roy. Soc.* 66: 488-489.
- Macrae, R. 1937. Interfertility phenomena of the American and European forms of *Panus stipticus* (Bull.) Fries. *Nature, Lond.* 139: 674.
- Macrae, R. 1942. Infertility studies and inheritance of luminosity in *Panus stipticus*. *Canad. J. Res.* 20: 411-434.
- McAlpine, D. 1901. Phosphorescent fungi in Australia. *Proc. Linn. Soc. N.S.W.* 25: 548-558.
- McAtee, W. L. 1947. Luminosity in Birds. *Amer. Midl. Nat.* 38: 207-215.
- McDermott, F. A. 1910-17. Observations on the light emission of American Lampyridae. *Canad. Ent.* 42: 357-363, 1910; 43: 399-406, 1911; 44: 73, 309-312, 1912; 49: 53-61, 1917.
- McDermott, F. A. 1910. The light of the firefly. *Elect. World N.Y.* 56: 1189.
- McDermott, F. A. 1911. Luciferescence, the fluorescent material present in certain luminous insects. *J. Amer. chem. Soc.* 33: 410-416.
- McDermott, F. A. 1911. The stability of the photogenic material of the Lampyridae and its probable chemical nature. *J. Amer. chem. Soc.* 33: 1791-1796.
- McDermott, F. A. 1911. Recent advances in our knowledge of the production of light in living organisms. *Smithson. Rep.*: 345-362. Also: *Sci. Amer., Suppl.*, 1911, No. 1842: 250-251.

- McDermott, F. A. 1911. Some observations on a photogenic microorganism, *Pseudomonas lucifera*, Molish. Proc. biol. Soc. Wash. 24: 179-184.
- McDermott, F. A. 1911. The "eye-spots" of *Alaus oculatus*. Canad. Ent. 43: 190-192.
- McDermott, F. A. 1911. Some considerations concerning the photogenic function in marine organisms. Amer. Nat. 45: 118-122.
- McDermott, F. A. 1914. The fire-fly and other luminous organisms. Trans. Illum. Engng. Soc. N.Y. 9: 413.
- McDermott, F. A. 1914. The ecologic relations of the photogenic function among insects. Z. wiss. Insekt Biol. 10: 303-307.
- McDermott, F. A. 1915. Experiments on the nature of the photogenic processes in the Lampyridae. J. Amer. chem. Soc., 37: 401-404.
- McDermott, F. A. 1916. Flashing of fireflies. Science 44: 610.
- McDermott, F. A. 1948. The common fireflies of Delaware. Pamphlet. Wilmington, Del., 18 pp.
- McDermott, F. A., and H. S. Barber. 1914. Luminous earthworms in Washington D.C. Proc. biol. Soc. Wash. 27: 145-150.
- McDermott, F. A., and C. G. Crane. 1911. A comparative study of the structure of the photogenic organs of certain American Lampyridae. Amer. Nat. 45: 306-313.
- McDermott, F. A., and H. E. Ives. 1914. The watts per candle efficiency of the fire-fly. Lighting J., N.Y. 1914: 61.
- McElroy, W. D. 1943. The application of the theory of absolute reaction rates to the action of narcotics. J. cell. comp. Physiol. 21: 95-116.
- McElroy, W. D. 1944. On the specificity of sulphanilamide action. J. cell. comp. Physiol. 23: 109-112.
- McElroy, W. D. 1944. The effect of narcotics and other inhibitors on the oxidation and assimilation of glucose by the luminous bacterium, *Achromobacter fisheri*. J. cell. comp. Physiol. 23: 171-192.
- McElroy, W. D. 1947. The energy source for bioluminescence in an isolated system. Proc. nat. Acad. Sci., Wash. 33: 342-345.
- McElroy, W. D. 1947. The mechanism of inhibition of cellular activity by narcotics. Quart. Rev. Biol. 22: 25-58.
- McElroy, W. D., and R. Ballentine. 1944. The mechanism of bioluminescence. Proc. nat. Acad. Sci., Wash. 30: 377-382.
- McElroy, W. D., and J. Coulombre. 1951. Purification and properties of adenosine triphosphate-light emitting system. Fed. Proc. 10: 219.
- McElroy, W. D., and A. H. Farghalv. 1948. Biochemical mutants affecting the growth and light production in luminous bacteria. Arch. Biochem. 17: 470-480.
- McElroy, W. D., and E. N. Harvey. 1951. Differences among species in the response of fire-fly extracts to adenosine triphosphate. J. cell. comp. Physiol. 37: 1-7.
- McElroy, W. D., and D. M. Kipnis. 1947. The mechanism of inhibition of bioluminescence by naphthoquinones. J. cell. and comp. Physiol. 30: 359-380.
- McElroy, W. D., and C. S. Rainwater. 1948. Spectral energy distribution of the light emitted by fire-fly extracts. J. cell. comp. Physiol. 32: 421-425.
- McElroy, W. D., and B. L. Strehler. 1949. Factors influencing the response of the bioluminescent reaction to adenosine triphosphate. Arch. Biochem. 22: 420-433.

- McElroy, W. D., J. Coulombre, and R. Hays. 1951. Properties of firefly pyrophosphatase. *Arch. Biochem. Biophys.* 32: 207-215.
- McIntosh, W. C. 1872. On the Abyssal theory of light, the Protozoic Absorption theory, and the Azoic-Mud theory, propounded in the reports of H. M. S. Porcupine 1869 and 1870. *Ann. Mag. Nat. Hist. (Ser. 4)* 9: 1-13.
- McIntosh, W. C. 1886. Phosphorescence of marine animals, a presidential address. Rep. B.A.A.S. held at Aberdeen 1885, 55: 1043-1053; *Rev. sci., Paris* (1885) 36 (Ser. 3) 10: 545-552; *Nature, Lond.* 32: 476-81 (1885).
- McIntosh, W. C. 1906. Photogenic marine animals. *Zoologist* (4) 10: 1-20.
- McKenney, R. E. B. 1902. Observations on the conditions of light production in luminous bacteria. *Proc. biol. Soc. Wash.* 15: 213-234.
- Maille, A. 1826. Note sur les habitudes naturelles des larves de Lampyres. *Ann. Sci. nat.* 7: 353-356.
- Mann, H., and R. A. R. Priske. 1912. Glow-worm larvae reared from eggs. *Proc. S. Lond. ent. nat. Hist. Soc.* 1911-12: 92.
- Majima, R. 1931. Studies on luminous bacteria. I. Luminous bacteria from *Microstomus kitaharae*, Jordan and Starks. *Sei-i-Kwai med. J.* 50: 1-23. Japanese with English summary.
- Majima, R. 1931. Studies on luminous bacteria. II. A study on photogenic bacteria establishing their saprophytic abode in flatfish procured in the market. *Sei-i-Kwai med. J.* 50: 33-40. In Japanese with English summary.
- Majima, R. 1931. Studies on luminous bacteria. III. On the fermentation of carbohydrates by various luminous bacteria and the immune-agglutination of the organisms. *Sei-i-Kwai med. J.* 50: 41-67. (Japanese with English summary.)
- Maluf, N. S. R. 1937, 39. Biology of Light Production in Arthropods. *Sci. Progr. Twent. Cent.* 32: 228-245. Also in *Smithson. Rep.* 1938: 377-404, 1939.
- Maluf, N. S. R. 1938. The basis of the rhythmic flashing of the firefly. *Ann. Ent. Soc. Amer.* 31: 374-380.
- Mangold, E. 1907. Ueber das Leuchten der Tiefseefische. *Pflüg. Arch. ges. Physiol.* 119: 583-601.
- Mangold, E. 1907. Leuchtende Schlangensterne und die Flimmerbewegung bei *Ophiopsila*. *Pflüge. Arch. ges. Physiol.* 118: 613-640. Also in *Biol. Zbl.* 28: 169-176, 1908.
- Mangold, E. 1910. Die Produktion von Licht. *Handb. vergl. Physiol.* 3 (2d half): 225-392. Jena.
- Mangold, E. 1912. Tierisches Licht in der Tiefsee. *Meeresk. in Vortr.* 6, Heft 8: 1-30.
- Mangold, E. 1925. Chemie der Lichtproduktion durch Organismen. *Handb. Biochem. Arbeitsmethoden*, Jena 2nd ed. 2: 433-441.
- Mangold, E. 1928. Die Produktion von Lichtenergie bei Tieren. *Handb. norm. pathol. Physiol.* 8 (2) 1072-1082.
- Mann, B. P. 1875. Notes on the luminous larvae of Elateridae. *Psyche, Camb., Mass.* 1: 89-93.
- Marazziti, V. 1937. Sull' antagonismo Ca-Mg e sull' azione dell'acido ossalico sui batteri luminosi. *Atti Ist. Veneto (Pt. 2)* 96: 93-104.
- Marquart. 1885. *Histoire Naturelle des Insects. "Suites a Buffon."* Vol. II, p. 497, on diptera.
- Mangold, E. 1940. Die Verwendung tierischen Lichts. *Rohstoffe des Tierreichs* Lief. 15, Band II, 414-416.

- Maslennikowa, W. A. 1927. Über biologische Eigenschaften der cholera-ähnlichen Vibrionen. Zbl. Bakt. (Abt. 1) 102: 148-157.
- Massart, J. 1893. Sur l'irritabilité des Noctiluques. Bull. sci. Fr. Belg. 25: 59-76.
- Mast, S. O. 1912. Behavior of fire-flies (*Photinus pyralis*?) with special reference to the problem of orientation. J. Anim. Behav. 2: 256-272. Also Science 35: 460.
- Matsubara, K. 1938. Studies on the deep-sea fishes of Japan, VI-VIII. J. Fish. Inst. Tokyo 33: 37-66.
- Matteucci, C. 1843. On the phosphorescence of the *Lampyris italica*. Ann. Mag. nat. Hist. 12: 373-374; C. R. Acad. Sci. Paris 17: 309-312, 1843. Ann. Chim. (Phys.) (Ser. 9) 3: 71.
- Matteucci, C. 1847. Lectures on the physical phenomena of living beings. London, 435 pp. Trans. by J. Pereira. Sect. VIII Phosphorescence of organized beings, pp. 161-185.
- Matzdorff, C. 1893. Ueber in Berlin angefundene leuchtende Regenwürmer. S.B. Ges. naturf. Fr. Berl. 1893: 19-23.
- Mehta, Dev Raj. 1932. Fauna of Lahore. 3. Preliminary notes on the life-history of the firefly *Luciola gorhami*. Bull. Dep. Zool. Panjab Univ. 1: 101-118.
- Meinken, H. 1934. Leuchtaugenfische. Aquarium, Berl., 87-90.
- Meissner, G. 1926. Bakteriologische Untersuchungen über die symbiontischen Leuchtbakterien von Sepien aus dem Golf von Neapel. Zbl. Bakt. (Abt. 2) 67: 194-238.
- Meissner, O. 1907. Wie leuchten die Lampyriden? Ent. Wbl. 24: 61.
- Meixner, J. 1933-36. Coleoptera. Leucht-Organ. In Kükenthal-Krumbach Handbuch der Zoologie, Vol. IV/2, pt. 1, pp. 1167-1173.
- Meldola, R. 1884. Phosphorescence of the Jelly-fish. Nature, Lond. 30: 289.
- Menge, F. A. 1856. Lebenszeichen vorweltlicher im Bernstein eingeschlossener Thiere, 32 pp. Progr. öffentl. Prüfung Schüler, Danzig, p. 21.
- Metcalf, M. M. 1918. The Salpidae, a taxonomic study. Bull. U.S. Nat. Mus. No. 100, 2: 1-193.
- Metcalf, M. M., and H. S. Hopkins. 1919. Pyrosoma, a taxonomic study based upon the collections of the U.S.B.F. and the U.S.N.M. Bull. U.S. Nat. Mus. No. 100, 2: 195-272.
- Metcalf, R. L. 1943. The isolation of a red-fluorescent pigment, lampyrine, from the lampyridae. Ann. ent. Soc. Amer. 36: 37-40.
- Meyen, F. J. F. 1834. Über das Leuchten des Meeres und Beschreibung einiger Polypen und anderer niederer Tiere. Nova Acta Leop. Carol. supp. 16: 125-218.
- Meyer, A. 1929. Tomopteris anadyomene nov. sp. ein Nachweis phylogenetische Umwandlung von Nephridialtrichtern in Leuchtorgane bei den Polydactelen. Zool. Anz. 86: 124-133.
- Meyer, K. P. 1942. Nachweis und Messung geringer Konzentrationen an freiem Sauerstoff (bis $1:10^{10}$) mittels Leuchtbakterien. Helv. phys. Acta 15: 3-22.
- Meyer, W. T. 1906. Ueber das Leuchtorgan der Sepiolum. Zool. Anz. 39: 388-392.
- Meyer, W. T. 1908. Ueber das Leuchtorgan der Sepiolum. II. Das Leuchtorgan von Heteroteuthis. Zool. Anz. 32: 505-508.
- Meyrick, E. 1886. A luminous insect larva in New Zealand. Ent. Month. Mag. 22: 266-267.
- Michaelis, G. A. 1830. Über das Leuchten der Ostsee nach eigener Beobachtungen. Hamburg, 52 pp.

- Migula, W. 1897. *System der Bakterien*. 2 vols. Phosphorescenz vol. I. 335-342. Jena.
- Miller, H. A. H. Farghaly, and W. D. McElroy. 1949. Factors influencing the recovery of biochemical mutants in luminous bacteria. *J. Bact.* 57: 595-602.
- Milne-Edwards, H. 1863. *Leçons sur la Physiologie et l'Anatomie comparée de l'Homme et des Animaux*. Paris, 8: (68 leçon), pp. 93-120. Production de lumière par les animaux.
- Mitchell, P. C. 1910-1926. Phosphorescence in zoology. *Encycl. Britann.*, 11th, 12th and 13th ed. 21: 476-478.
- Mitchill, S. L. 1801. Luminous appearance of ocean water caused by animals. *Phil. Mag.* 10: 20-27.
- Möbius, K. 1878. Die Bewegung der fliegenden Fische durch die Luft. *Zeit. wiss. Zool.*, 30: suppl.: 343-382.
- Moelwyn-Hughes, E. A. 1937. The kinetics of enzyme reactions. (4.) The oxidation of luciferin, catalysed by luciferase. *Ergebn. Enzymforsch.* 6: 32-35.
- Molisch, H. 1902. Ueber Heliotropismus im Bakterienlichte. *S.B. Akad. Wiss. Wien*, 111 (Abt. 1): 141-148.
- Molisch, H. 1903. Bakterienlicht und photographische platte. *S.B. Akad. Wiss. Wien* 112 (Abt. 1): 297-316.
- Molisch, H. 1903. Über das Leuchten des Fleisches, insbesondere toten Schlachtthiere. *Bot. Ztg.* 61 (Abt. 1): 1-18.
- Molisch, H. 1904. Die Leuchtbakterien des Hafens von Triest. *S.B. Akad. Wiss. Wien* 113 (Abt. 1) 513-527.
- Molisch, H. 1904. Leuchtende Pflanzen. Eine physiologische Studie, Jena. Second ed. 1912.
- Molisch, H. 1904. Ueber Kohlensäuren-Assimilations-Versuche mittelst der Leuchtbakterienmethode. *Bot. Ztg.* 62 (Abt. 1): 1-10.
- Molisch, H. 1905. Luminosity in Plants. *Rep. Smithson. Instn.* 351-362.
- Molisch, H. 1905. Ueber das Leuchten von Hühnereiern und Kartoffeln. *S.B. Akad. Wiss. Wien* 114 (Abt. 1): 3-14.
- Molisch, H. 1907. *Lafar's Handbuch der technischen Mykologie*. (Gustav Fischer), Jena. 3rd ed., Photogene Bakterien 1: 623-640.
- Molisch, H. 1908. Ueber einige angeblich leuchtende Pilze. *Wiesner Festschr.*, Wien, 19-23.
- Molisch, H. 1925. Botanische Beobachtungen in Japan. III. Über das leuchten des schlacht-viehfleisches in Sendai, Japan. *Sci. Rep. Tōhoku Univ. (Ser. 4) Biology*. 1: 97-103; also *Pflanzenbiol. in Japan*, Jena, 1926.
- Molisch, H. 1925. Über Kohlensäure-Assimilation toter Blätter. *Z. Bot.* 17: 577-593.
- Moniez, R. 1888-1889. Note sur le Lumbricus (Photodrilus) phosphoreus Duges. *Rev. Biol. Nord Fr.* 1: 197-200.
- Monzette, G. F. 1921. Luminous beetles of Florida. *Florida Ent.* 4: 17-18.
- Moore, A. R. 1924. Luminescence in Mnemiopsis. *J. gen. Physiol.* 6: 403-412; also *Proc. Soc. exp. Biol.* 21: 52-54.
- Moore, A. R. 1925. The inhibition of luminescence by light—Dynamics of the reaction. *Proc. Soc. exp. Biol. N.Y.* 23: 6-7.
- Moore, A. R. 1925. (1) The inhibition of luminescence by light. (2) Electrical stimulation of luminescence—a case of reversed Pflüger's Law. *Amer. J. Physiol.* 72: 214-215, 230.

- Moore, A. R. 1926. On the nature of inhibition in *Pennatula*. Amer. J. Physiol. 76: 112-115.
- Moore, A. R. 1926. The photolysis of the luminescent granules of *Eucharis multicornis*. J. gen. Physiol. 8: 4, 303-310.
- Moore, A. R. 1926. Galvanic stimulation of luminescence in *Pelagia noctiluca*. J. gen. Physiol. 9: 375-81.
- Moore, A. R. 1926. Inibizione della luminescenza nei Ctenofori. Arch. Sci. Biol. 8: 112-121.
- Moore, A. R. 1926. On the ionic basis of electrical stimulation. Proc. Soc. exp. Biol., N.Y. 23: 341-342.
- Moore, B. 1908. Observations on certain organisms of (a) variations in reaction to light and (b) a diurnal periodicity of phosphorescence. Bio-chem. J. 4: 1-29.
- Moore, H. B. 1950. The relation between the scattering layer and the Euphariscea. Biol. Bull. Wood's Hole 99: 181-212.
- Moore, N. B. 1873. (Habits and light of *Pyrophorus physoderus* compared with those of *P. noctilucus* and *Photinus pyralis*.) Rep. Com'p Agric. Wash., Rep. Entomol. 1873: 154-155.
- Morley, C. 1896. Glowworms in October. Entomologist 29: 64-65.
- Morley, C. 1901. Note on the pairing of *Lampyrus noctiluca*. L. Ent. mon. Mag. 37: 226.
- Morrison, T. F. 1925. Studies on luminous bacteria. II. The influence of temperature on the intensity of the light of luminous bacteria. J. gen. Physiol. 7: 741-753.
- Morrison, T. F. 1925. The effect of polarized light on the growth of luminous bacteria. Science 61: 392-393.
- Morrison, T. F. 1929. Observations on the synchronous flashing of fireflies in Siam. Science 69: 400-401.
- Mortara, S. 1917. Nouvelle recherche sulla *Pyroteuthis margaritifera* Hoyle over sulla *Carybditeuthis maculata* Vir. Mem. R. Com. talassogr. ital. 57: 3-28.
- Mortara, S. 1917. Galliteuthis armata Joubin e Galliteuthis philura Berry. Mem. R. Com. talassogr. ital. 40: 3-6.
- Mortara, S. 1918. La disposizione degli organi ciatiformi del genere *Aplya* e suoi rapporti con quella del genere *Gobius*. Mem. R. Com. talassogr. ital. 45: 5-23.
- Mortara, S. 1921. Gli organi luminosi di *Pyroteuthis margaritifera* e le loro complicazioni morfologiche. Mem. R. Com. talassogr. ital. 82: 1-30.
- Mortara, S. 1922. Ancora sulla biofotogenesi. R. C. Accad. Lincei (Ser. 5), 31: 54-58.
- Mortara, S. 1922. Sulla biofotogenesi. Nota preliminare. R. C. Accad. Lincei (Ser. 5), 31: 187-190.
- Mortara, S. 1922. È accettabile la teoria simbiotica della fotogenesi animale. Rivista Biol. 4: 1-2.
- Mortara, S. 1922. Gli organi fotogeni di *Abralia veranyi*. Mem. R. Com. talassogr. ital. 95: 1-18.
- Mortara, S. 1924. Sulla biofotogenesi e su alcuni batteri fotogeni. Riv. Biol. 6: 323-342.
- Moseley, H. N. 1877. On the coloring matter of various animals and especially of deep-sea forms dredged by H. M. S. Challenger. Quart. J. Micro. Sci. 2: 1-23.
- Moseley, H. N. 1879. Notes by a naturalist on the Challenger. London, 620 pp. pp. 574 and 590 for phosphorescent animals.

- Mosely, H. N. 1887. Report on the structure of the peculiar organs on the head of *Ipnots*. *Challenger Rep.* 22: 269-276.
- Moufflet, A. 1865. No title [*Phosphorescence du Fulgora lanternaria* L.]. *Ann. Soc. ent. Fr.* (Ser. 4), 5: *Bull.* p. LXII.
- Mudrak, A. 1933. Beiträge zur Physiologie der Leuchtakterien. *Zbl. Bakt.* (Abt. 2) 88: 353-366.
- Müller, G. W. 1891. Neue Cypridiniden. *Zool. Jb.* (Abt. 1) 5: 211-252.
- Müller, J. 1862. Phosphoreszenz eines Seefisches. *S.B. Ges. naturf. Fr. Berl.* Mar. 18, 1862.
- Müller, O. F. 1806. *Zoologia danica seu animalium daneae et norvegicae. Havniae.* Vol. 4, p. 31.
- Muir, F. 1913-1917. [On *Fulgora*] Presidential address. *Proc. Hawaii. ent. Soc.* 3: 28-42.
- Mulder, E. 1860. Natürliches und künstliches Phosphorescence von Fischen. *Arch. Holland Beitr. Natur u. Heilk. Utrecht* 2: 398-407 and *Nederl. Gast. Ooglijd. Versl. (Donders)* 2: 398-407.
- Mulder, E. 1861. Note sur la phosphorescence naturelle et artificielle des poissons. *Ann. Sci. nat. (Zool.)* (Ser. 4) 15: 367 and *J. Physiol.* 4: 234-241.
- Muraoka, H. 1896. Das Johanniskäferlicht. *Ann. Phys. Lpz., N.F.* 59: 773-781; also *J. Coll. Sci. Tokyo* 1897, 9: 129-139.
- Muraoka, H., and M. Kashva. 1898. Das Johanniskäferlicht und die Wirkung der Dämpfe von festen und flüssigen Köpern auf photographische Platten. *Ann. Phys. Lpz.* 64: 186-192.
- Murray, A. 1870. On an undescribed light giving Coleopterous larva (provisionally named *Astraptor illuminator*). *J. linn. Soc.* 10: 74-82.
- Murray, J. 1826. Experimental researches on the light and luminous matter of the glow-worm, the luminosity of the sea, the phenomena of the Chameleon, the ascent of the spider into the atmosphere, and the torpidity of the tortoise, etc., Glasgow, 177 pp.
- Murray, J. 1876. II Preliminary reports to Prof. Wyville Thomson FRS, Director of the Civilian Staff, on work done on board the "Challenger." *Proc. roy. Soc.* 24: 451-544.
- Murray, J., and J. Hjort. 1912. *The Depths of the Ocean.* London, 821 pp.
- Murray, J., and W. Thomson. 1885. *Narrative of the cruise of the Challenger, 1: 222.*
- Murrill, W. A. 1915. Luminescence in the Fungi. *Mycologia* 7: 131-133.
- Murrill, W. A. 1920. Plant growths that shed light. *Sci. Amer.* 122: 427, 440.
- Nadson, G. 1903. Sur la phosphorescence des bacteries. *Bull. Jard. bot. St.-Petersb.* 3: 110-123. In Russian with French summary.
- Nadson, G. 1908. Zur Physiologie der Leuchtakterien. I. Die Bedeutung der Salze im Nahsubstrat. II. Das Leuchten der Photobakterien in der Symbiose mit anderen Microorganismen. *Bull. Jard. bot. St.-Petersb.* 8: 144-158. In Russian with German summary.
- Naef, A. 1921. Das System der dibranchiaten Cephalopoden usw. *Mitt. zool. Sta. Neapel* 22: 527-542.
- Naef, A. 1923. Die Cephalopoden. *Fauna u. Flora Neapel.* 35 Monog. 1st Part Systematik Berlin 148 pp. 2nd part Embryologie, 1928.
- Nakamura, H. 1939. Hydroperoxid und bakterielles Leuchten. *Acta phytochim., Tokyo* 11: 159-166.
- Nakamura, H. 1940. Über die Hydrogenlyase und die Hydrogenase in Leucht-

- bakterien, nebst einer Bemerkung über die Möglichkeit ihrer Beteiligung an Leuchtvorgang. *Acta phytochim.*, Tokyo 11 (2): 239-247.
- Nakamura, H. 1940. Light production by bacteria. *Bot. Zool.* 8: 154, 701-710. In Japanese.
- Nakamura, H. 1940. Ueber die Bedeutung der Hydrogenation bei Biolumineszenz. *Bot. Mag. Tokyo* 54: 314-318. Japanese, with German summary.
- Nakamura, H., and Y. Fukumura. 1940. Methylene blue and luminescent bacteria. *Kagaku (Science)*, Japan 10: (13): 462-463.
- Nakamura, H. 1942. Über das Leuchten der Leuchtbakterien bei Zugabe von verschiedenen Oxydation-Reduktionsindikatoren. *Bot. Mag. Tokyo* 56: 456-460. In Japanese with German summary.
- Nakamura, H. 1942. Zur Kenntnis der leuchtenden Dinoflagellaten in Japan. *Bot. Mag. Tokyo* 56: 553-554. In Japanese with German summary.
- Nakamura, H. 1942. Co-luciferase in luminous bacteria and fungi. *Med. Biol.* 2 (6): 318-320. In Japanese.
- Nakamura, H. 1942. Cold Light. Kobundo, Tokyo, 139 pp. In Japanese.
- Nakamura, H. 1944. Luminescent micro-organisms. Tokyo, 197 pp. In Japanese.
- Nakamura, H. 1947. Ueber die Bedeutung von Flavin bei der Biolumineszenz. *Bot. Mag. Tokyo* 60: 703-714. In Japanese with German summary.
- Nakamura, H., H. Satomi, Y. Sueno, and M. Yamamoto. 1940. Physiologisch-chemische Studien über das Leuchten des Lebewesens. I. *Bot. Zool.* 8: 1423-5. In Japanese with German summary.
- Naudin. 1846. Phosphorescence des feuilles sèches. *Rev. hort.*, Paris (Ser. II). 254.
- Neumann, G. 1934. Tunicata acopa-caducichordata. Pyrosomida. *Kükenthal-Krumbach Handb. Zool. Berlin* 5: 203-323. Especially pp. 225-229 and 256-268.
- Neush, J. 1879. Bactéries lumineuses sur la viande fraîche. *Journ. Pharm. Chim.* Paris 29: 20-22; also *Bull. Sci. Dept. Nord et Rev. internat. Sci.* Oct. 1878.
- Newall, R. S. 1879. Snails v. Glow-worms. *Nature, Lond.* 20: 197, 220, 243.
- Newman, E. 1864. Luminosity of the Lantern-fly. *Entomologist*, 2: 22-26.
- Newport, G. 1845. Monograph of the class myriapoda, Order Chilopoda. *Trans. Linn. Soc. Lond.* 19: 349-438.
- Newport, G. 1857. On the natural history of the glow-worm (*Lampyris noctiluca*). *Proc. Linn. Soc. London*, 1: 40-71.
- Nichols, E. L. 1924. The brightness of marine luminescence. *Science* 60: 592-593. See also 55: 157-9, 1922.
- Niedermeyer, A. 1911. Studien über den Bau von *Pteroides griseum* (Bohadsh). *Arb. zool. Inst. Univ. Wien* 19: 99-164.
- Niemann, W. 1925. Eigenartiges meeresleuchten im Indischen Ozean. *Ann. Hydrogr. Berlin* 53: 398-399.
- Ninomiya, R. 1924. Der Einfluss von Antikörpern und Komplement auf biologische Functionen von Bakterien. I. Der Einfluss spezifischer Amboceptoren mit und ohne Komplementzusatz auf das Leuchtvermögen von Leuchtbakterien. *Z. Immun.Forsch.*, 39: 498-512.
- Nishikawa, T. 1901. *Gonyaulax* and the discolored water in the bay of Agu. *Annot. zool. jap.* 4: 31-34.
- Nobecourt, P. 1926. Sur l'*Armillaria mellea* Vahl en cultures pures. *Assoc. Fr. Av. Sc.* (50 sess.) Lyon, 365-369.
- Noll, F. 1879. Einige Beobachtungen im Seewasser-Zimmeraquarium. *Zool. Anz.* 2: 402-405, 455.

- Noll, F. 1885. Meine Reise nach Norwegen im Sommer 1884. Ber. Senckenberg. Naturforsch. Ges. Frankfurt a.M. 1886. Anhang 1-42.
- Norman, J. R. 1930. Oceanic fishes and flatfishes collected in 1925-1927. Discovery Rep. 2: 261-370.
- Norris, A. 1894. Observations on the New Zealand glow worm, *Bolitophila luminosa*. Ent. mon. Mag. 30: 202-203.
- Nowikoff, M. 1930. Das Prinzip der Analogie und die Vergleichende Anatomie. 185 pp. Jena. Die Formen der Leuchtorgane, 123-144.
- Nuesch, J. 1877. Ueber das leuchtende Fleisch gestorbener Tiere. Kosmos 1, and Gaea, Köln 13: 549.
- Nusbaum-Hilarowicz, J. 1923. Etudes d'anatomie comparée sur les poissons provenant des campagnes scientifiques de S.A.S. le Prince de Monaco. Result. Camp. Sci. Monaco, Pt. I Fasc. 58, 115 pp.; Pt. II Result, Fasc. 65, 100 pp.
- Nuttall, G. H. F. 1923. Symbiosis in animals and plants. Nature, Lond. 112: 657-660.
- Nutting, C. C. 1899. The utility of phosphorescence in deep-sea animals. Amer. Nat. 33: 792-799.
- Nutting, C. C. 1907. The theory of abyssal light. Proc. 7th Internat. Zool. Cong. Boston, pp. 889-899.
- Obaton, F. 1938. Mesure de la brillance des Photobacterium en fonction de leur développement. C. R. Acad. Sci., Paris 206: 1504-1506.
- Obaton, F. 1939. Influence de la colchicine sur la developement de Photobacterium phosphoreum. C. R. Acad. Sci., Paris 208: 1536-1538.
- Obaton, F. 1943. La phosphorescence de Clitocybe olearia (Fries et de Candolle). Bull. Soc. bot. Fr. 90: 213-215.
- Ohshima, H. 1911. Some observations on the luminous organs of fishes. J. Coll. Sci. Tokyo 27: Art. 15, 1-25.
- Okada, Y. K. 1925. Luminescence in sponges. Science 62: 566-567.
- Okada, Y. K. 1926. On the photogenic organ of the knightfish (*Monocentris japonicus* (Houttuyn)). Biol. Bull. Wood's Hole 50: 365-373.
- Okada, Y. K. 1926. Light localization in Ctenophores. Science 63: 262.
- Okada, Y. K. 1927. Contribution à l'étude des céphalopodes lumineux. Notes preliminaries. I. and II. Bull. Inst. océanogr. Monaco, No. 494, 1-16; No. 499, 1-15.
- Okada, Y. K. 1927. Luminescence et organe photogène des Ostracodes. Bull. Soc. zool. Fr. 51: 478-486.
- Okada, Y. K. 1927. Luminescence chez les molluscs lamellibranches. Bull. Soc. zool. Fr. 52: 95-98.
- Okada, Y. K. 1928. Notes on the tail organs of Asctes. Ann. Mag. nat. Hist. (Ser 10) 1: 308.
- Okada, Y. K. 1928. Two Japanese Aquatic Glowworms. Trans. R. ent. Soc. Lond. 76: 101-108.
- Okada, Y. K. 1935. Origin and development of the photogenic organs of Lampyrids with special reference to those of *Luciola cruciata* and *Pyrocoelia rufa*. Mem. Coll. Sci. Kyoto 10B: 209-228.
- Okada, Y., and K. Baba. 1938. On the luminous organs of a nudibranch *Plocamophorus tilesii*. Berge. Annot. zool. jap., 17: 276-281.
- Okada, Y., and K. Kato. 1949. Studies on luminous animals in Japan. III. Preliminary report on the life history of *Cypridina hilgendorfi*. Bull. biogeogr. Soc. Japan 14 (3): 21-25, a translation from Kagaku 16: 64-66, 1946.

- Okada, Y., S. Koisi, and I. Yasuda. 1937. Über die Lichtproduction des Leuchtkäfers. *Mitt. med. Akad. Kioto* 19: 1325-1327.
- Okada, Y. K., S. Takagi, and H. Sugino. 1933. Microchemical studies on the so-called photogenic granules of *Watasenia scintillans* (Berry). *Proc. imp. Acad. Japan* 10: 431-434.
- Okochi, N. 1949. Culture of luminescent bacteria (IV-VI). *Jour. Agric. Soc. Japan* 22: 131-140.
- Omeliarsky, W. L. 1911. Die Einwirkung der Radiumstrahlen auf die leuchtenden Bakterien. *Z. Balneol.* 4: 405-408.
- Orioli, F. 1850. De' pesci e del mare che rilucono nella oscurità. *Ann. Sci. Mat. Fis. (Tortolini)* 1: 242-247.
- Osorio, B. 1912. Une propriété singulière d'une bactérie phosphorescente. *C. R. Soc. Biol. Paris* 72: 432-433.
- Osorio, T. B. F. 1943. El Mar de Cortes y la productividad fitoplanctonica de sus aguas. *An. Esc. Nac. Cienc. Biol. Mexico*, 3: 73-118.
- Osten-Sacken, C. R. von. 1861. Die Amerikanischen Leuchtkäfer. *Stettin. ent. Ztg.* 22: 54-56.
- Osten-Sacken, C. R. von. 1862. Unknown larvae. *Proc. ent. Soc. Philadelphia* 1: 125-130.
- Osten-Sacken, C. R. von. 1862. Characters of the larvae of mycetophilidae. *Proc. ent. Soc. Philadelphia* 1: 151-172.
- Osten-Sacken, C. R. von. 1878-9. Luminous insects, especially diptera. *Ent. mon. Mag.* 15: 43-44.
- Osten-Sacken, C. R. von. 1886. A luminous insect larva from New Zealand. *Ent. mon. Mag.* 22: 266; 23: 99-101, 133; 24: 230.
- Ostroumoff, A. 1924. *Noctiluca miliaris* in Symbiosis mit grünen Algen. *Zool. Anz.* 58: 162.
- Owens, H. B. 1944. Notes on a degenerate female of the genus *Phengodes*. *Bull. nat. Hist. Soc. Maryland* 13: 76-77.
- Owsjannikow, P. 1864. Über das Leuchten der Larven von *Lampyrus noctiluca*. *Bull. Acad. Sci. St.-Petersb.* 7: 55-61.
- Owsjannikow, P. 1868. Ein Beitrag zur Kenntnis der Leuchtorgane der *Lampyrus noctiluca*. *Mem. Acad. Sci. St. Petersburg. (Ser. 7)* 11: No. 17, 1-11.
- Packard, A. S. 1896. The phosphorescent organs of insects. *J. N.Y. ent. Soc.* 4: 61-66.
- Packard, A. S. 1898. A textbook of entomology. The phosphorescent organ. pp. 424-429, N.Y.
- Panceri, P. 1870, 1871. Intorno ad una forma non per anco notata negli zooidi delle pennatule. *R.C. Accad. Sci. Napoli* 9: 23-28; 10: 113-115.
- Panceri, P. 1871. Intorno alla luce emanata dal grasso. *R. C. Accad. Sci. Napoli* 10: 79-81; also *Ann. Chim. appl. Roma* (1872).
- Panceri, P. 1871. Intorno alla sede del movimento luminoso nelle meduse. *R. C. Accad. Sci. Napoli* 10: 140-146.
- Panceri, P. 1872. The luminous organs and light of the pennatulæ. *Quart. J. micr. Sci.* 12: 248-254.
- Panceri, P. 1872. The light organs and the light of the Pholades. *Quart. J. micr. Sci.* 12: 254-259.
- Panceri, P. 1872. Gli organi luminosi e la luce delle Foladi. *R. C. Accad. Napoli* 11: 83-88.

- Panceri, P. 1872. Intorno ad un Pennatulario fosforescenti non peranco rinvenuto presso Napoli. *R. C. Accad. Sci. Napoli* 11: 88-90.
- Panceri, P. 1872. La luce e gli organi luminosi dei Beroidei. *R. C. Accad. Sci. Napoli* 11: 172-173.
- Panceri, P. 1872. La luce degli occhi farfalle. *R. C. Accad. Sci. Napoli* 11: 213-218.
- Panceri, P. 1872. Études sur la phosphorescence des animaux marines. *Ann. Sci. nat. (Zool.)* 16: (Ser. 5), No. 8, p. 68.
- Panceri, P. 1873. Gli organi luminosi e la luce delle pennatule. *Atti Accad. Sci. fis. mat. Napoli* 5 (No. 10): 1-38.
- Panceri, P. 1873. Gli organi luminosi e la luce dei Pirosomi e della Foladi. *Atti Accad. Sci. fis. mat. Napoli* 5 (No. 13): 1-51.
- Panceri, P. 1873. Intorno alla luce che emana delle cellule nervosi della *Phyllirrhoe bucephala*. *Atti Accad. Sci. fis. mat. Napoli* 5 (No. 14): 1-12. Also *Quart. J. micr. sci.* 13 NS.: 109-116.
- Panceri, P. 1873. La luce e gli organi luminosi dei Beroidei. *Atti accad. Sci. fis. mat. Napoli* 5: (No. 20) 1-15.
- Panceri, P. 1873. The luminous organs and light of *Pyrosoma*. *Quart. J. micr. Sci.* 13: 45-51.
- Panceri, P. 1874. Intorno alla luce che emana dai nervi delle elitre della *Polynoe*. *R. C. Accad. Napoli* 13: 143-147.
- Panceri, P. 1875. La luce e gli organi luminosi di alcuni anelidi. *R. C. Accad. Napoli* 14: 21-25.
- Panceri, P. 1877. Luminous *Campanulariae*. *Nature, Lond.* 16: 30.
- Panceri, P. 1878. La luce e gli Organi luminosi di alcuni anelidi. *Atti Accad. Sci. fis. mat. Napoli* 7 (No. 1): 1-20.
- Panceri, P. 1878. Appendice relativa ad una ofiura luminosa. *Atti Accad. Sci. fis. mat. Napoli* 7: No. 1, 17.
- Panceri, P. 1878. Intorno alla sede del movimento luminoso nelle *Campanularie*. *Atti Accad. Sci. fis. mat. Napoli* 7 (No. 9): 1-6.
- Parfitt, E. 1880. On the phosphorescence of the glow-worm. *Ent. mon. Mag.* 17: 94.
- Parker, G. H. 1920. The Phosphorescence of *Renilla*. *Proc Amer. phil. Soc.* 19: 171-175.
- Parker, G. H. 1920. Activities of colonial animals. II. Neuromuscular movements and phosphorescence of *Renilla*. *J. exp. Zool.*, 31: 475-513.
- Parker, H. W. 1939. Luminous organs in lizards. *J. Linn. Soc. (Zool.)* 40: 658-660.
- Parlin, W. A. 1935. How bright is a lightning-bug? *Sci. Amer.* 153: 15.
- Parr, A. E. 1927. Ceratioidea. Scientific results of the third oceanographic expedition of the "Pawnee." *Bull. Bingham oceanogr. Coll.* 3 (Art. 1) 34 pp.
- Parr, A. E. 1927. Stomioid fishes of the suborder Gymnophotodermi from the waters around the Bahama and Bermuda Islands. *Bull. Bingham oceanogr. Coll.* 3 (Art. 2) 123 pp.
- Parr, A. E. 1928. Deep-sea fishes of the order Iniomi from the waters around the Bahama and Bermuda islands. With annotated keys to the Sudidae, Myctophidae, Scopelarchidae, Lycerhamulidae, Omosudidae, Celomimidae and Rondeletidae of the world. *Bull. Bingham oceanogr. Coll.*, 3 (Art. 3), 193 pp.
- Parr, A. E. 1929. Notes on the species of myctophine fishes represented by type

- specimens in the United States National Museum. *Proc. U.S. nat. Mus.* 76: Art. 10, 1-47.
- Parr, A. E. 1930. On the probable identity, life history and anatomy of free-living and attached males of the ceratioid fishes. *Copeia* 129-135.
- Parr, A. E. 1931. Deep-sea fishes from off the western coast of North and Central America. *Bull. Bingham oceanogr. Coll.*, 2, art. 4, 53 pp.
- Parr, A. E. 1934. Report on experimental use of a triangular trawl for bathypalagic collecting with an account of the fishes obtained and a revision of the family Cetomimidae. *Bull. Bingham oceanogr. Coll.*, 4 (Art. 6) 59 pp.
- Parr, A. E. 1937. Concluding report on fishes. *Bull. Bingham oceanogr. Coll.*, 5, art. 7, 79 pp.
- Parr, A. E. 1946. The Macrouridae of the western North Atlantic and Central American seas. *Bull. Bingham oceanogr. Coll.* 10, Art. 1, 99 pp.
- Passerini, N. 1882. Sull' organo ventrale del *Geophilus gabrielis*. *Ricerche sulla pelle dei myriapoda*. *Boll. Soc. ent. ital.* 14: 323-328.
- Pasteur, L. 1864. Sur la lumière phosphorescente des Cucujos. *C. R. Acad. Sci. Paris* 59: 509-510.
- Patouillard, N. 1882. Observations sur quelque hymenomycetes. *Bull. Soc. mycol.* 4: 208-211. Abstract in *J. R. micr. Soc.* 1883: 106.
- Patouillard, N. 1887. Champignons de la Nouvelle-Calédonie. *Bull. Soc. mycol. Fr.* 3: 168-178.
- Patten, W. 1886. Eyes of molluscs and arthropods. *Mitt. zool. Sta. Neapel* 6: 542-756.
- Paucă, M. 1929. Vorläufige Mitteilung über eine fossile Fischfauna aus den Oligozänschiefern von Suslanesti (Muscel). *Bul. Acad. roman Sect. Sti.* 12: 112-120.
- Paucă, M. 1931. Neue Fische aus dem Oligozän von Piatra-Neamt. *Bul. Acad. roman Sect. Sti.* 14: 29-34. Also in *C. R. Inst. géol. Roum.* 19: 89-97, 1933.
- Peach, C. W. 1850. Observations on the luminosity of the sea, with descriptions of several of the objects which cause it, some new to the British coast. *Ann. Mag. Nat. Hist.* 6 (Ser. 2): 425-434.
- Peattie, D. C. 1949. The miracle of the fire-fly. *Reader's Dig.* 28: 86-88.
- Pelvet, M. 1867. Note sur quelques phénomènes de phosphorescence chez des animaux marins. *C. R. Soc. Biol. Paris (Ser. 4)*, 4: 23-27.
- Penners, A. 1931. Die Leuchtorgane. In Bolk, Göppert, Kallius and Lubosch. *Handb. vergl. Anat. Wirbeltiere. I*: 693-702.
- Peragallo. 1862. Note pour servir a l'histoire des Lucioles. *Ann. Soc. ent. Fr.* 2: (Ser. 4) 620-624; 3: 661-665, 1863.
- Percheron, A. R. 1835. Note sur trois insectes lumineux. *Rev. Ent. Strassburg et Paris* 3: 76.
- Perkins, G. A. 1869. The Cucuyo; or West Indian Fire Beetle. *Amer. Nat.* 2: 422-433.
- Perkins, M. 1931. Light of glow worms. *Nature, Lond.* 128: 905.
- Péron, F. 1804. Mémoire sur le nouveau genre *Pyrosoma*. *Ann. Mus. Hist. Nat. Paris* 4: 437-446. Also in *J. Med.*, 10: 88-89 (1805); *J. Phys.*, 59: 207-213; Voigt. *Magazin f. de neueste Zustand der Naturkunde* 9: 3-12, 1805.
- Péron, F. 1804. Sur la température des eaux de la mer, soit à sa surface, soit à diverses profondeurs, le long des rivages et en pleine mer. *Ann. Mus. Hist. nat. Paris* 5: 123-148. Also in *Phil. Mag.* 21: 129-133 (1805).
- Péron, F. 1807. Voyage de decouvertes aux terres australes. Vol. 1, 496 pp.

- Péron, F., and C. A. Lesueur. 1809. Histoire generale et particulière de tous les animaux qui composent la famille des Meduses. *Ann. Mus. hist. nat. Paris* 14: 218-228. See also 15: 41-56, 1810.
- Perrier, E. 1886. Les explorations sous-marines. Paris, 352 pp.
- Peters, A. W. 1841. Über das Leuchten der *Lampyrus italica*. *Arch. Anat. Physiol., Lpz.* 229-233; and *Ann. Sci. nat.*, 17 (Ser. 2): 254, a translation.
- Peters, A. W. 1905. Phosphorescence in Ctenophores. *J. exp. Zool.* 2: 103-116.
- Peters, N. 1926. Noctiluca mit grünen Symbionts. *Zool. Anz.*, 67: 193-194.
- Pethen, R. W. 1913. Glow worms and lightning. *Knowledge* 36: 13-14.
- Pflaff, C. H. 1828. Bemerkungen über das Leuchten des Meeres. *J. von Chem. Phys.* 22: 311-318.
- Pfeffer, G. 1912. Die Cephalopoden der Plankton Expedition. Zugleich eine monographische Übersicht der Oegopsiden Cephalopoden. *Ergebn. Plankton Exped. Humboldt-Stiftung*, 2, F.a.
- Pfeffer, W. 1906. The Physiology of Plants, vol. 3. 2nd ed. revised, edited and trans. by A. J. Ewart. The Production of Light, pp. 382-388.
- Pfeiffer, H., and H. J. Stammer. 1930. Pathogenes Leuchten bei Insecten. *Z. Morph. Ökol. Tiere* 20: 136-171.
- Pflüger, E. 1875. Die Phosphorescence lebendiger Organismen und ihre Bedeutung für die Principien der Respiration. *Pflüg. Arch. ges. Physiol.* 10: 275-300.
- Pflüger, E. 1875. Ueber die Phosphorescenz verwesender Organismen. *Pflüg. Arch. ges. Physiol.* 11: 222-263.
- Phillips, W. 1888. La luminosité des champignons (tr). *Rev. mycol.* 10: 120-125.
- Phipson, T. L. 1858. De la phosphorescence en général et des insectes phosphoriques. *J. Med. Chir. Pharmacol. Brussels* 26: 101-106, 200-205, 302-308.
- Phipson, T. L. 1860. Sur la matière phosphorescente de la raie. *C. R. Acad. Sci. Paris* 51: 541-542.
- Phipson, T. L. 1869. Phosphorescence; or, the emission of light by minerals, plants and animals. New York and London, 210 pp.
- Phipson, T. L. 1872. Sur la noctilucine. *C. R. Acad. Sci.* 75: 547; also *Chem. News*, 25: 130, 1872.
- Phipson, T. L. 1876. On Noctilucine the phosphorescent principle of luminous animals. *J. Franklin Inst.* 101: (Ser. 3) 71: 68-72. Also *Chem. News* 32: 221-230, 1875.
- Pickering, W. H. 1916. Photometry of West Indian Fire-fly. *Nature, Lond.* 97: 180.
- Pickford, G. E. 1937. A monograph of the acanthodriline earthworms of South Africa. Cambridge, England. Phosphorescence, pp. 40-41, 112, 403.
- Pickford, G. E. 1939. The Vampyromorpha. A new order of dibranchiate Cephalopoda. *Vestn. čsb. Zool. Spolec.* 7: 346-358.
- Pickford, G. E. 1946, 1949. *Vampyrotheuthis infernalis* Chun, an archaic dibranchiate cephalopod. I Natural history and distribution; II External anatomy. Dana Report Nos. 29 and 32 Carlsberg Foundation's Oceanographic Expedition 1928-1930, 40 pp. and 132 pp.
- Pickford, G. E. 1950. The Vampyromorpha (Cephalopoda) of the Bermuda oceanographic expeditions. *Zoologica, N.Y.* 35 (part 1): 87-95.
- Pierantoni, U. 1914. La luce degli insetti luminosi e la simbiosi ereditaria. *R. C. Accad. Napoli* 20: 15-21.
- Pierantoni, U. 1914. Sulla luminosita e gli organi luminosi di *Lampyrus noctiluca*. *Boll. Soc. Nat. Napoli* 27: 83-88.

- Pierantoni, U. 1918. Organi luminosi, organi simbiotici e glandola nidamentale accessoria nei Cefalopodi. *Boll. Soc. Nat. Napoli* 30: 30-36.
- Pierantoni, U. 1918. I microorganismi fisiologica e la luminescenza degli animali. *Scientia, Bologna* 23: 102-110.
- Pierantoni, U. 1918. Gli organi simbiotici e la luminescenza batterica dei Cefalopodi. *Pubbl. Sta. zool. Napoli* 2: 105-146.
- Pierantoni, U. 1919. La simbiosi fisiologiche e le attivita dei plasmi cellulari. *Riv. Biol.* 1: 213-221.
- Pierantoni, U. 1920-23. Gli organi luminosi simbiotici ed il loro circolo ereditario in *Pyrosoma giganteum*. *Pubbl. Sta. zool. Napoli* 3: 191-222.
- Pierantoni, U. 1920. Per una piu esatta conoscenza degli organi fotogeni dei cefalopodi abissali. *Arch. zool. (ital.) Napoli* 9: 195-213.
- Pierantoni, U. 1920. Sul significato fisiologico della simbiosi ereditaria. *Boll. Soc. Nat. Napoli* 33: 55-66.
- Pierantoni, U. 1921. Organi luminosi batterici nei pesci. *Riv. Biol.* 3: 342-346.
- Pierantoni, U. 1921. Note di morfologia e sviluppo sui fotofori degli Eufausiacei. *Pubbl. Sta. zool. Napoli* 3: 165-186.
- Pierantoni, U. 1922. Simbiosi e biofotogenesi. *R. C. Accad. Lincei* 31: 385.
- Pierantoni, U. 1923. I recenti ricerche sulla simbiosi fisiologica ereditaria. *Arch. Sci. biol. Napoli* 4: 229-237. Also *Atti Soc. ital. Progr. Sci.* 13: 1-20, 1924.
- Pierantoni, U. 1923. L'organo dorsale del *Pyrosoma giganteum*. *Pubbl. Sta. zool., Napoli, Memoria* 4: 1-12.
- Pierantoni, U. 1923. Nuove osservazioni su luminiscenza e simbiosi I La fosforescenza degli *Oligochaeti*. *R. C. Accad. Lincei. (Ser. 5)* 32: 359-362.
- Pierantoni, U. 1924. I recenti studii sulla simbiosi fisiologica ereditaria. *Atti Soc. ital. Progr. Sci.* 13: 1-20.
- Pierantoni, U. 1924. La fosforescenza e la simbiosi in *Microsculex phosphoreus*. *Boll. Soc. Nat. Napoli* 36: 179-195.
- Pierantoni, U. 1924. Nuove osservazioni su luminescenza e simbiosi. II. La fosforescenza dei *Ctenophori*. *R. C. Accad. Lincei (Ser. 5)* 33: 241-244.
- Pierantoni, U. 1924. Nuove osservazioni su luminescenza e simbiosi. III. Organo luminoso di *Heteroteuthis dispar*. *R. C. Accad. Lincei (Ser. 5)* 33: 61-65.
- Pierantoni, U. 1925. I microrganismi nell' economia animale. *Scientia, Bologna* 37: 243-252.
- Pierantoni, U. 1926. Ancora sulla bioluminescenza da simbiosi. *Riv. Biol.* 8: 241-4.
- Pierantoni, U. 1927. I corpuscoli fotogeni di *Heteroteuthis dispar*. *Boll. Soc. Nat. Napoli* 38: 3-7.
- Pierantoni, U. 1929. Gli organi fotogeni a batterii dei pesci. *Riv. Fis. Mat. Sci. Nat.* 3 (Ser. IIa): 3-9.
- Pierantoni, U. 1931. Nuovo rivenimento di Pesci con organi luminosi e batteri. *Riv. Fis. Mat. Sci. Nat.* 5: 257-258.
- Pierantoni, U. 1936. Gli studii sulla endosimbiosi ereditaria nelle origini e nei piu recenti sviluppi. *Attual. zool.* 2: 137-195. Supplement to *Arch. Zool. Ital.*
- Pierantoni, U. 1939. Le radiazioni luminescenti di origine biologica (biofotogenesi). *Trattato Radiobiol.* 3: 1-85.
- Pieron, H. 1925. La persistence a l'obscurite du rythme lumineux du *Lampyre*. *Feuill. jeun. Nat.* 186-188.
- Piffard, B. 1864. Reminiscence of an entomological excursion up the Danube river. *Ent. mon. Mag.* 1: 79-81, 104-107.

- Plate, L. 1906. *Pyrodinium bahamense* n.g.n.sp., die Leuchtperidinacee des "Feuersees" von Nassau, Bahamas. Arch. Protistenk 7: 411-427.
- Plate, L. 1888. Studien über Protozoen. VII Bemerkungen über *Noctiluca miliaris* und das durch sie hervorgerufene meerleuchten. Zool. Jb. Abt. 2, 3: 174-180. Trans. in Ann. Mag. Nat. Hist. (Ser. 6) 3: 22-28, 1889.
- Polimanti, O. 1911. Über das Leuchten von *Pyrosoma elegans* Les. Z. Biol. 55: 505-529.
- Potts, F. A. 1913. The swarming of *Odontosyllis*. Proc. Camb. phil. Soc. 17: 193-200.
- Pouchet, G. 1882. Sur l'évolution des Peridiniens et les particularités d'organisation, que les rapprochent des Noctiluques. C. R. Acad. Sci., 95: 794-796.
- Pouchet, G. 1883. Contribution à l'histoire des cilioflagellates. J. Anat. Paris 19: 399-455.
- Pouchet, G. 1885. Nouvelle contribution à l'histoire des Peridiniens marins. J. Anat. Paris 21: 28-88; 525-534; 23: 87-112, 1887.
- Pouchet, G. 1888. De la multiplication provoquée et de la forme de Noctiluques. C. R. Soc. Biol. Paris (Ser. 8) 5: 575-576.
- Pouchet, G. 1889. Du cytoplasme et du noyau chez les Noctiluques. C. R. Acad. Sci. Paris 89: 706-707.
- Pouchet, G. 1889. De la structure et des phénomènes nucléaires chez les Noctiluques. C. R. Soc. Biol. Paris (Ser. 9) 1: 642.
- Pouchet, G. 1890. Contribution à l'histoire des noctiluques. Journ. Anat. Paris 26: 104-125.
- Prashad, B. 1923. Observations on the luminosity of some animals in the Gangetic Delta. J. Asiat. Soc. Beng. N.S. 18: 581-584.
- Pratje, A. 1921. Das Leuchten der Tiere. Naturw. Wschr., 20: 433-440.
- Pratje, A. 1921. *Noctiluca miliaris*, Beiträge zur Morphologie, Physiologie u. Cytologie. I. Morphologie u. Physiologie. Arch. Protistenk. 42: 1-95.
- Pratje, A. 1921. Zur Chemie der *Noctiluca* Zellkernes. Beiträge zur Morphologie, Physiologie u. Cytologie. II. Z. Anat. EntwGesch. 62: 171-232.
- Pratje, A. 1921. Macrochemische, quantitative Bestimmung des Fettes u. Cholesterins, sowie ihren Kennzahlen bei *Noctiluca miliaris*, Sur. Biol. Zbl. 41: 433-446.
- Pratje, A. 1921. Die verwandtschaftlichen Beziehungen der Cystoflagellaten zu den Dinoflagellaten. Arch. Protistenk. 42: 423-438.
- Pratje, A. 1923. Das Leuchten der Organismen. Eine Übersicht über die neuere Literatur. Ergebn. Physiol. 21: 1-108.
- Pratje, A. 1925. *Noctiluca*. Tierwelt N. u. Ostsee. G. Grimpe and E. Wagler, Part II, d₁-d₁₂.
- Pratje, A. 1931. Lichterzeugung durch Organismen. Handwörterbuch der Naturwiss., 6: 283-296.
- Prince, E. E. 1892. *Maurolicus borealis*, Nilss. One of the British phosphorescent fishes. Trans. nat. Hist. Soc. Glasg. 3: lxxxiii-lxxxiv.
- Pring, J. H. 1849. Observations and experiments on the *Noctiluca miliaris*, the animalcular source of the phosphorescence of the British seas; together with a few general remarks on the phenomena of vital phosphorescence. Phil. Mag. 34 (Ser. 3) 401-421. Also in Rep. Brit. Ass. 19th meeting p. 81, 1849.
- Priske, R. A. R., and H. Main. 1911. Notes on the glow-worm (*Lampyris noctiluca*). Proc. S. Lond. ent. nat. Hist. Soc. 1910-1911, 74-76.

- Prochnow, O. 1905. Lichtstärke von *Lampyrus noctiluca*, L. Ent. Zeit. 19: 173-174.
- Pryer, W. B. 1880. Tropical notes (observations on fireflies). Ent. mon. Mag. 17: 241-245.
- Pütter, A. 1905. Leuchtende Organismen. Z. allg. Physiol., Sammelref. 5: 17-53.
- Pütter, A. 1911. Vergleichende Physiologie. Jena, 721 pp. Die Production strahlender Energie, pp. 481-488.
- Pütter, A. 1912. Lichtproduction durch Organismen. Handwörterbuch der Naturwiss., 6: 333-340, 509.
- Puisségur, C. 1946. Les Êtres Vivants Lumineux. Sci. et Vie 70, No. 348, Sept.
- Puntoni, V. 1925. Lo stato attuale della teoria microbica della biophotogenesi. Riv. Biol., 7: 150-157.
- Quatrefages, A. de. 1843. Note sur un nouveau mode de phosphorescence observé chez quelques Annelides et Ophiures. Ann. Sci. nat. Zool. (Sér. 2) 19: 183-192.
- Quatrefages, A. de. 1850. Mémoire sur la phosphorescence du port de Boulogne et sur les animaux qui la produisent. C. R. Acad. Sci. Paris 31: 428, 618-621.
- Quatrefages, A. de. 1850. Mémoire sur la phosphorescence de quelque invertébrés marins. Ann. Sci. nat. Zool. (Sér. 3) 14: 226-235. Translated in Amer. J. Sci. 15: 193-203 and 16: 69-78, 1853.
- Quatrefages, A. de. 1850. Observations sur les noctiluques. Ann. Sci. nat. Zool. (Sér. 3) 14: 236-281.
- Quatrefages, A. de. 1862. The phosphorescence of the sea. Pop. Sci. Rev. 1: 275-298.
- Quoy, J. R. C., et J. P. Gaimard. 1825. Observations sur quelques Mollusques et Zoophytes, envisagés comme causes de la Phosphorescence de la mer. Ann. Sci. Nat. Zool. (Sér. 1) 4: 5-13.
- Quoy, J. R. C., et J. P. Gaimard. 1827. Description des genres Biphore, Carinaire, Hyale, Fleche, Cléodore, Anatife et Briorée. Ann. Sci. nat. Zool. 10: 225-239.
- Raffenau-Delile, A. 1833. Description de l'Agaric de l'olivier (*Agaricus olearius*), et examen de sa phosphorescence. Guillemain Arch. Bot. 2: 519-527.
- Raj, J. S. 1943. A note on the egg-laying habits of the Indian glow-worm (*Lamprophorus tenebrosus* Wlk.). J. Bombay nat. Hist. Soc. 43: 675-678; 44: 142-143, 1944.
- Raj, J. S. 1943. On the external morphology of the larva of the glow-worm, *Diaphanes* sp. (Lampyridae: Col.). Curr. Sci. 12: 276-278.
- Rake, G., H. Jones, and C. M. McKee. 1943. Antiluminescent activity of antibiotic substances. Proc. Soc. exp. Biol. 52: 136-138.
- Rake, G., C. M. McKee, and H. Jones. 1942. A rapid test for the activity of certain antibiotic substances. Proc. Soc. exp. Biol. 51: 273-274.
- Ramadan, M. 1938. On luminosity in Penaeidae, with a description of the photophores of *Hymenopenaeus debelis*. Sci. Rep. John Murray Exped. Brit. Mus. (1933-34), 5, Appendix 1, 137-140.
- Ramdas, L. A., and L. P. Venkiteswaran. 1931. The spectrum of a glowworm (*Lampyridae*). Nature, Lond. 128: 726-727.
- Rang, S. 1829. Etablissement de la famille des Béroïdes dans l'ordre des Acalèphes libres, et description de quelques nouveaux genres qui lui appartiennent. Bull. Univ. Sci. Sec. 2, 17: 142.
- Rapp. 1828. Untersuchungen über den Bau einige Polypen des Mitteländischen Meeres. Nova Acta Leop. Carol. 14: 643-658.

- Rathke, H. 1835. Beschreibung der *Oceania Blumenbachii*. Akademia nauk. Leningrad. Mem. Acad. Sci. St. Petersburg. (Div. Sav.) (Ser. 6), 2: 321-329. Also Arch. Naturgesch. 11 (1): 117-9.
- Rathvon, S. S. 1870. Luminous (?) leaf hopper. Amer. Entom. Bot. 2: 371.
- Rau, P. 1932. Rhythmic periodicity and synchronous flashing in the fire fly, *Photinus pyralis*, with notes on *Photuris pennsylvanica*. Ecology 13: 7-11.
- Rauffer, M. 1927. Die Leuchtorgane. Bronns Klassen und Ordnung des Tierreiches., 6, Abt. 1, Book 2, 125-167. Leipzig.
- Rawitz, B. 1892. Der Mantelrand der Acephalen. Jena. Z. Naturw. (N.S.) 20: 1-232.
- Ray, C. N. 1945. Phosphorescent Texas Earthworms. Science 101: 271-272.
- Ray, D. L. 1950. The peripheral nervous system of *Lampanyctus leucopsarus*. J. Morph., 87: 61-178.
- Regan, C. T. 1912. Classification of the teleostian fishes of the order Pediculati. Ann. Mag. nat. Hist. (Ser. 8), 9: 277-289 (Ser. 9), 15: 561-567, 1925.
- Regan, C. T. 1923. The classification of the stomiatoid fish. Ann. Mag. nat. Hist. (Ser. 9), 11: 612-614.
- Regan, C. T. 1926. The pediculate fishes of the suborder Ceratoidea. Danish "Dana" Exped. (1920-22) Rep. No. 2, pp. 1-45.
- Regan, C. T., and E. Trewavas. 1929. Astronesthidae and Chauliodontidae. Danish "Dana" Exped. 1920-22, Rep. No. 5.
- Regan, C. T., and E. Trewavas. 1930. Stomiatidae and Malacosteidae. Danish "Dana" Exped. 1920-22, Rep. No. 6.
- Regan, C. T., and E. Trewavas. 1932. Deep sea angler fishes. Danish Dana Exped. Rep. No. 2, 113 pp.
- Reiche, L. 1849. [Details sur la crepitation du *Brachinus africanus* et sur la lueur phosphorescente qui l'accompagne.] Ann. Soc. ent. Fr. (Ser. 2) 7, Bull. 60.
- Reichensperger, A. 1908. Ueber Leuchten von Schlangensterne. Biol. Zbl., 28: 166-169.
- Reichensperger, A. 1908. Die Drüsengebilde der Ophiuren. Zeit. wiss. Zool. 91: 304-350.
- Reinelt, J. 1906. Beiträge zur Kenntnis einiger Leuchtbakterien. Zbl. Bakt. (Abt. 2), 15: 289-300.
- Reinhardt, J. 1854. Tvende Jagttaglser of phosphorisk Lysning hos en Fisk og en Insectlarve. Videnskabeliger Medeleser 60-65; Trans. R. ent. Soc. Lond. (Ser. 2a) 3, Proc. 5-8; also in Z. Naturwiss 5: 208-213 (1855).
- Reinhardt, J. T. 1854. Note on a luminous fish (*Astronesthes fieldii*). Zoologist 12: 4299-4300.
- Reinke, J. 1898. Ueber das Leuchten von *Ceratum tripes*. Wiss. Meeresunters., herausgeg. v.d. Kommis. z. wiss. Unters. deutsch. Meers in Kiel u.d. Biol. Sta. Helgoland, N.F. 3, Abt. Kiel, 37-41.
- Reinking, O. A. 1921. The synchronal flashing of fire-flies. Science 53: 485-486.
- Reitz, A. 1909. Leuchtbakterien. Mikrokosmos 3: 1-5.
- Rerabek, J., and D. E. Hykesova. 1937. Wirkung der Radiumemanation auf das Leuchten des Protoplasmas. (Versuche mit dem Leuchtbacterium, *Vibrio phosphorescens*.) Protoplasma 28: 360-364.
- Richard, J. 1885. Un mot sur la phosphorescence des Myriapodes. Ann. Soc. ent. Belg. 29: 15-20.
- Richmond, C. A. 1930. Fireflies flashing in unison. Science 71: 537-538.

- Richter, O. 1906. Chlorophyllbildung im Bakterienlichte. S.B. Akad. Wiss. Wien 115: 298-300.
- Richter, O. 1926. Bakterienleuchten "ohne Sauerstoff." *Planta* 2: 569-587.
- Richter, O. 1928. Natrium ein notwendiges Nährelement für eine marine micro-ärophile Leuchtbacterie. Denkschr. Akad. Wiss. Wien 101: 261-292; also Anz Akad. Wiss. Wien 65: 163-165.
- Ridley, H. N. 1936. The luminous secretion of the centipede *Geophilus* as a defense against the attack of beetles, etc. *Proc. R. Ent. Soc. Lond. A* 11: 48.
- Ries, J. von, and M. von Ries-Imchanitzky. 1940. Lichttod und Lichtausstrahlung. *Radiolog. Clin.* 9: 257-298.
- Riley, C. V. 1880. Large phosphorescent larva. "Answers to correspondents." *Amer. Entom.* 3: 201-202.
- Riley, C. V. 1887. Notes on *Phengodes* and *Zarhipis*. *Proc. ent. Soc. Wash.* 1: 62-63, 86-88; *Ent. mon. Mag.* 24: 148-149; *Nature, Lond.* 36: 592.
- Risbec, J. 1925. Production de lumière par un mollusque nudibranche de la Nouvelle Calédonie. *C. R. Acad. Sci. Paris* 181: 472-473.
- Risbec, J. 1928. Contribution a l'étude des nudibranches Néo-Calédoniens. *Faune Colon. franç.* 2 (fasc. 1), 205-211.
- Risso, A. 1810. *Ichthyologie de Nice ou histoire naturelle de poissons du département des alpes maritimes.* Paris, 388 pp., p. 55.
- Risso, A. 1820. Sur deux nouvelles espèces de poissons du genre *scopelus*, observés dans la mer de Nice. *Mem. R. Accad. Torino* 25: 262-272.
- Rivers, I. J. 1886. Descriptions of the form of the female in a Lampyrid (*Zarhipis* Riversi, Horn). *Amer. Nat.* 20: 648-650.
- Rizzuti, G. 1906. Azione della luce sulla fosforescenza dei batteri luminosi. *G. med. Eserc.* 54: 777-782.
- Roberts, F. M. 1916. Luminous centipedes. *Nature, Lond.* 98: 269.
- Robin, C. 1878. Recherches sur la reproduction geminipare et fissipare des Noctiluques. (*Noctiluca miliaris*.) *J. Anat. Paris*, 14: 563-629, and *C. R. Acad. Sci. Paris* 86: 1482-1486.
- Robin, C., et A. Laboulbène. 1873. Sur les organes phosphorescents thoraciques et abdominaux du Cocuyo de Cuba (*Pyrophorus noctilucus*). *C. R. Acad. Sci. Paris* 77: 511-517; also, *Ann. Soc. ent. Fr.* 3: 529-536, 1874; also, *Journ. Anat. Paris* 9: 593-600.
- Robin, C., et C. Legros. 1866. De l'action exercé par l'électricité sur les Noctiluces miliaries. *J. Anat.* 3: 558-559.
- Robineau-Desvoidy. 1849. Note sur le *Thyreophora cynophila*. *Ann. Soc. ent. Fr.* (Ser. 2) 7, Bull. V-VI.
- Robson, G. C. 1926. Light organs in littoral cephalopoda. *Nature, Lond.* 118: 554-5.
- Robson, G. C. 1932. A monograph on recent Cephalopoda based on the collections in the British Museum. II Octopoda (excluding the Octopodinae). 359 pp. London.
- Robson, G. C. 1948. The Cephalopoda Decapoda of the *Arcturus oceanographia* expedition, 1925. *Zoologica, N.Y.* 33: 115-132.
- Rogerson, W. 1821. On the glow worm. *Phil. Mag.* 58, 33; *Istis, Leipzig* 1818, cap. 5, p. 456.
- Roman, W. 1939. Luciferase. In C. Oppenheimer's *Die Fermente und ihre Wirkungen*, Supp. 2: 1646-1652.
- Root, C. W. 1932. The relation between respiration and light intensity of

- luminous bacteria, with special reference to temperature. I. Temperature and light intensity. *J. cell. comp. Physiol.* 1: 195-208.
- Root, C. W. 1934. The relation between respiration and light intensity of luminous bacteria with special reference to temperature. II. Temperature and oxygen consumption. *J. cell. comp. Physiol.* 5: 219-228.
- Rosenfeld, G. 1902. Studien über das Fett von Meeresorganismen. *Wiss. Meeresuntersuch.* N.F. 5, Abt. Helgoland, 57-83.
- Rottier, P. B. 1942. Fluorometrische en spectrophotometrische bepaling van lacto flavine in micro-organismen. *Dissert. Delft.*
- Roule, L. 1919. Poissons. *Res. Camp. sci. Monaco Fasc.* 52, 190 pp.
- Roule, L., and F. Angel. 1930. Larves et Alevins de Poissons. *Res. Camp. Sci. Monaco Fasc.* 79, 148 pp.
- Roule, L., and F. Angel. 1933. Poissons. *Res. Camp. Sci. Monaco Fasc.* 86, 168 pp.
- Roumeguere, C. 1882. Un nouvel agaric lumineux [*A. (Gymnopus) socialis* Fr.] signale par l'Abbé Dulac. *Rev. mycol.* 4: 10-13.
- Ruckmick, C. A. 1920. A possible interpretation of the synchronous flashing of fire-flies. *Trans. Ill. Acad. Sci.* 13: 103-122.
- Ruedemann, R. 1937. Observations on excitation of fireflies by explosions. *Science* 86: 222-223.
- Rüppell, E. 1844. Intorno ad alcuni Cefalopodi del mare di Messina. *G. Gabinetto mare Messina. Fasc.* 26, 129-135.
- Russell, H. L. 1892. Impfungsversuche mit Giard's Pathogenen *Leuchtbacillus*. *Zbl. Bakt.* 11: 557-559.
- Russell, W. J. 1897. On the action exerted by certain metals and other substances on a photographic plate. *Proc. roy. Soc.* 61: 424-433.
- Ryder, J. A. 1880. Phosphorescence of very young fishes. *Amer. Nat.* 14: 675-6.
- Rye, E. C. 1878. Luminous lepidopterous larvae. *Ent. mon. Mag.* 14: 257-260.
- Sanzo, L. 1912. Uova di Sternoptychidae. *Boll. b. Com. talassogr. ital.*, No. 27.
- Sanzo, L. 1912. Comparsa degli organi luminosi in una serie di larve di *Gonostoma denudatum*. *Mem. R. Com. talassogr. ital.* 9: 1-22.
- Sanzo, L. 1912. Larva di *Stomias boa* Risso. *Mem. R. Com. talassogr. ital.* 10: 3-7.
- Sanzo, L. 1913. Larva di *Ichthyococcus* (Cocco). *Mem. R. Com. talassogr. ital.* No. 27.
- Sanzo, L. 1913. Stadi post-embrionali di *Vinciguerria attenuata* (Cocco) e *V. Poveriae* (Cocco) Jordan ed Evermann. *Mem. R. Com. talassogr. ital.* 35: 3-8.
- Sanzo, L. 1914. Contributo alla conoscenza degli stadi larvali negli Scopelini Muller (*Bathophilus nigerrimus* Gigl., *Scopelus caninus* C. & V., *Sc. humboldti* Risso). *Mem. Accad. Lincei (Ser. 5)*, 10: 714-720.
- Sanzo, L. 1914. Stadi larvali di *Chauliodus. Sloani* Bl. *Mem. R. Com. talassogr. ital.* 39: 3-7.
- Sanzo, L. 1915. Contributo alla conoscenza dello sviluppo negli Scopelini Müller (*Saurus griseus* Lowe, *Chlorophthalmus Agassizii* Bp. ed *Aulopus filamentosus* Cuv.). *Mem. R. Com. talassogr. ital.* 49: 3-21.
- Sanzo, L. 1915. Stadi larvali di *Bathophilus nigerrimus* Gigl. *Mem. R. Com. talassogr. ital.* 48: 3-11.
- Sanzo, L. 1918. Contributo alla conoscenza dello sviluppo post-embrionate negli Scopelini Müller. *Mem. R. Com. talassogr. ital.* 66: 5-54.

- Sanzo, L. 1918. Nuovo contributo alla conoscenza della sviluppo larvale di *Stomias* boa Risso. R. C. Accad. Lincei, 27, sem. 2.
- Sanzo, L. 1928, 1930, 1935. Uova, sviluppo embrionale, stadi larvali, post-larvali e giovanili di Sternoptychidae e Stomiatidae. I. *Argyropelecus hemigymnus* Cocco II *Ichthyococcus ovatus* Cocco III *Maurolicus pennanti* Walb. Monogr. R. Com. talassogr. ital. 2: 1-181.
- Sanzo, L. 1931. Uova larva e stadi giovanili di Teleosti. Salmonoidei, Stomiatoidi. Flora e Fauna Golfo Napoli. Monog. 38, 21-92 and Macruridae 255-265 of *Hymenocephalus italicus*.
- Sanzo, L. 1932. Uova e larve di *Gonostoma denudatum* Rof. Boll. Zool. 3: 78-80.
- Sars, G. O. 1885. Report on the Schizopoda. Voyage of H. M. S. Challenger, 13: 255 pp., 70-72 on "luminous globules."
- Sars, G. O. 1907. Notes supplémentaires sur les Calanoidés de la Princesse-Alice. Bull. Inst. oceanogr. No. 101.
- Sars, G. O. 1925. Copepodes particulièrement bathypelagiques. Result. Camp. sci. Monaco 69: 408 pp. and 127 plates.
- Sars, M. 1864. Beskrivelse over den af ham i Aaret 1856 opstillede Thysanopoda norvegica. Forh. Vidensk Selsk. Krist. Aar 1863, 2-10; 79-84.
- Sasaki, M. 1914. Observations on Hotaru-ika *Watasenia scintillans*. J. Coll. Agric. Sapporo 6: 75-105.
- Schertel, S. 1902. Leuchtpilze, unsere gegenwärtige Kenntnis von ihnen, ihr Vorkommen in Literatur und Mythe. Deutsch bot. Monatsch. Armstadt, 22: 39-42, 56-60, 76-77, 139-152.
- Schiller, J. 1935-1937. Dinoflagellatae (Peridineae). L. Rabenhorst's Kryptogamen-Flora, Bd. X, 3 abt. 2 Hälfte, 484-493 on *Pyrocystis*.
- Schmid, C. A. 1803. Versuche über die Insecten, Gotha, 1: 233, 245.
- Schmidt, J. 1922. Live specimens of *Spirula*. Nature, Lond. 110: 788-790.
- Schmidt, P. 1894. Über das Leuchten der Zuckmücken (Chironomidae). Zool. Jb. Abt. Syst., 8: 58-66. Transl. in Ann. Mag. Nat. Hist. (6) 15: 133-141.
- Schmidt, P. J. 1930. On some rare Japanese fishes and fishes new for Japan. Proc. 4th Pac. Sci. Congr. Java Batavia-Bandoeng Vol. III Biol. paper pp. 459-466.
- Schmitz, J. von. 1843. Über den Bau, das Wachstum und einige besonderen Lebenserscheinungen der *Rhizomorpha fragilis*. Linnaea 1: 487-535.
- Schnauss, J. 1862. Bericht über meine Versuche, die chemische Wirkung des Lichtes von *Lampyrus* nachzuweisen. Nova Acta Leop.-Carol. 30: 114-116.
- Schneyer, L. H. 1951. The effect of low concentrations of calcium and phosphate salts on bacterial luminescence intensity. J. cell. comp. Physiol. 37: 337-350.
- Schoepfle, G. M. 1940. Kinetics of luminescent flashes in the bacterium, *Achromobacter fischeri*, at different temperatures. J. cell. and comp. Physiol. 16: 341-360.
- Schoepfle, G. M. 1941. Kinetics of bacterial luminescent flashes: Effects of veronal, dinitrophenol, and osmotic pressure. J. Cell. Comp. Physiol. 17: 109-116.
- Schreiber, E. 1927. Die Reinkultur von marinem Phytoplankton und deren Bedeutung für die Erforschung der Produktionsfähigkeit des Meerwassers. Wiss. Meeresunters. N.F. 10, No. 10, 1-34.
- Schubert, H. 1934. Beobachtungen an Leuchtbakterien. 17 p. 8° Rostock.
- Schultz, I. P. 1938. Review of the fishes of the genera *Polyipnus* and *Argyropelecus* (Family Sternoptichidae), with descriptions of three new species. Proc. U.S. nat. Mus. 86 (No. 3047), 135-155.

- Schultz, O. 1899. Phosphorescierende Lichterscheinung an den antennen von *Asteroscopus sphinx* Hufn. (*Cassinia* F.). Berlin ent. Z. 44: 319-320.
- Schultze, M. 1864. Über den Bau der Leuchtorgane der Männchen von *Lampyrus splendidula*. Sitzber. niederhein. Ges. Natur. u. Heilk. zu Bonn, p. 61; also in Arch. Naturgesch. 30: 363-365.
- Schultze, M. 1865. Zur Kenntniss der Leuchtorgane von *Lampyrus splendidula*. Arch. mikr. Anat. 1: 124-137.
- Schultze, M. 1886. Kleine Mittheilungen. Beobachtungen an *Noctiluca*. Arch. mikr. Anat., 2: 163-165.
- Schurig, W. 1901. Biologische Experimente nebst einem Anhang: Microscopische Technik. Leipzig.
- Scudder, S. H. 1895. The miocene insect fauna of Oeningen, Baden. Geol. Mag., Lond. N.S. 2: 116-122.
- Seaman, W. H. 1891. On the luminous organs of insects. Proc. Amer. Soc. Microscopists 13: 133-162.
- Secchi, P. 1872. Nouvelles observations sur les lumieres phosphorescentes animales. Ann. Sci. Nat. Zool. (Ser. 5) 16, article 9.
- Seifter, J. 1945. An unusual action of amphetamine. Science 102: 597.
- Severn, H. A. 1881. Notes on the Indian glow-fly. Nature, Lond. 24: 165.
- Shapiro, H. 1934. The light intensity of luminous bacteria as a function of oxygen pressure. J. cell. comp. Physiol. 4: 313-327.
- Shepherd, J. 1894. An account of phosphorescence caused by an ostracod. Vict. Nat. 11: 131.
- Shibita, E., T. Takeda and T. Inoue. 1936. Eine neue Theorie über das Leuchten der leuchtenden Organismen. Plants and Animals 4: 1323-1329.
- Shima, G. 1927. Preliminary note on the nature of the luminous bodies of *Watasenia scintillans* Berry. Proc. imp. Acad. Tokyo 3: 461-464.
- Shoji, R. 1919. A physiological study on the luminescence of *Watasenia scintillans* (Berry). Amer. J. Physiol. 47: 534-557.
- Shoup, C. S. 1928. Preservation of luminous bacteria in absence of oxygen. Proc. Soc. exp. Biol. 25: 570-572.
- Shoup, C. S. 1929. The respiration of luminous bacteria and the effect of oxygen tension upon oxygen consumption. J. gen. Physiol. 13: 27-45.
- Shoup, C. S. 1930. The luminous bacteria. J. Tenn. Acad. Sci. 5: 1-4.
- Shoup, C. S. 1933. Luminescence and respiration of bacteria in carbon monoxide. Biol. Bull. Wood's Hole 65: 370.
- Shoup, C. S. and A. Kimler. 1934. The sensitivity of the respiration of luminous bacteria for 2,4-dinitro-phenol. J. cell. comp. Physiol. 5: 269-275.
- Shoup, C. S. 1941. Luminescence and oxygen consumption of *Photobacterium fischeri* in oxygen-carbon monoxide mixtures. Yearb. Amer. Philos. Soc. 1941, 190-191.
- Silvester, C. F., and H. W. Fowler. 1926. A new genus and species of phosphorescent fish. *Kryptophanaron alfredi*. Proc. Acad. nat. Sci. Philad. 78: 245-247.
- Sinclair, F. G. 1895. Myriapoda, in Camb. Nat. Hist. 5: 29-80. New York.
- Singh, P., and S. Maulik. 1910. Nature of light emitted by fire-flies. Nature, Lond. 99: 111.
- Skowron, S. 1926. On the luminescence of *Microsclex phosphoreus* Dug. Biol. Bull. Wood's Hole 51: 199-208.
- Skowron, S. 1926. On the luminescence of some cephalopods (*Sepiola* and *Heteroteuthis*) Riv. Biol. 8: 236-240.

- Skowron, S. 1928. The luminous material of *Microscolex phosphoreus* Dug. *Biol. Bull. Wood's Hole*, 54: 191-195.
- Skowron, S. 1928. Über das Leuchten des Tiefseefisches *Chauliodus Sloanii*. *Biol. Zbl.*, 48: 680-684.
- Skuse, F. A. A. 1890. Description of a luminous dipterous insect (fam. *Mycetophilidae*) from New Zealand. *Proc. Linn. Soc. N.S. W.* (2) 5: 677-679.
- Skuse, F. A. A. 1891. Description of *Bolitophila luminosa*, Skuse. *Trans. N. Z. Inst.* 23: 47-49.
- Smith, F. 1869. The larva of the *Pyrophorus* of Uruguay. *Proc. R. ent. Soc. Lond.* 15.
- Smith, H. M. 1935. Synchronous flashing of fireflies. *Science* 82: 151-152.
- Smith, W. G. 1871. Luminous fungi. *J. Bot. Lond.* 9: 176-177; *Gardener's Chron. Amer. p.* 1289, 1872.
- Snell, P. A. 1932. The control of luminescence in the male lampyrid firefly, *Photuris pennsylvanica*, with special reference to the effect of oxygen tension on flashing. *J. cell. comp. Physiol.* 1: 37-51. Also in *Science* 73: 372-373, 1931.
- Snyder, C. D., and A. H. Snyder. 1920. The flashing interval of fire-flies—Its temperature coefficient—An explanation of synchronous flashing. *Am. J. Physiol.* 51: 536-543.
- Söhngen, N. L. 1913. Einfluss von Kolloiden auf mikrobiologische Prozesse. *Zbl. Bakt. (Abt. 2)* 38: 621-647.
- Sokolow, I. 1909. Zur Frage über das Leuchten und die Drüsengebilde der Ophiuren. *Biol. Zbl.* 29: 637-648.
- Solger, B. 1881. Zur Kenntnis der Verbreitung von Leuchtorganen bei Fischen. *Arch. mikr. Anat.* 19: 147-152.
- Sonnenschein, C. 1931. Fortzüchtung von Leuchtvibrionen in Rindergalle. *Zbl. Bakt. (Abt. 1)* 123: 92-93.
- Sonnenschein, C. 1931. Leuchtvibrionen als Sauerstoffzähler bei der Anaerobenzüchtung und also Sauerstoffindikator. *Zbl. Bakt. (Abt. 1)* 123: 378-381.
- Sonnenschein, C. 1932. Auf Leuchtvibrionen wirksame Bakteriophage. *Zbl. Bakt. (Abt. 1)* 126: 297-302.
- Soru, E., and R. Braumer. 1932. Action at a distance of phosphorescent bacteria on bone marrow. *C. R. Soc. Biol. Paris* 111: 201-203.
- Spence, W. 1848. No title. On the luminosity of *Fulgora lanternaria*. *Trans. R. ent. Soc. Lond.* 5: proc. 38-39.
- Spiller, J. 1882. Spectrum of the light of the Glow-worm. *Nature, Lond.* 26: 343.
- Spinola, M. 1839. Essai sur les Fulgorelles, sous-tribu de la tribu des Cicadaïdes. ordre des Rhyngotes. *Ann. Soc. ent. Fr.* 8: 133-449.
- Spix, J. B. von, and C. F. P. von Martius. 1823. Reise in Brasilien 1817-20. München. Pt. I 1823; II 1828, III 1831. 1824. Travels in Brazil in the years 1817-20, London.
- Spruit, C. J. P. 1946. Naphthochinonen en bioluminescentie. Thesis. Utrecht. 150 pp. In Dutch with English summary.
- Spruit, C. J. P. 1947, 1948. Carbonyl-substituted naphthoquinones. I Methylketones unsubstituted in the side chain. II Ketones of the type $C_{10}H_7O_2-COCH_2R$. *Rec. Trav. chim. Pays-Bas* 66: 655-672; 67: 285-297.
- Spruit, C. J. P. 1949. The chemical nature of the luciferins. *Enzymologia* 13: 191-200.
- Spruit, C. J. P., and A. L. Schuiling. 1945. On the influence of naphthoquinones

- on the respiration and light emission of *Photobacterium phosphoreum*. *Reu. Trav. chim. Pays-Bas* 64: 220-228.
- Spruit, van der Burg, A. 1950. Emission spectra of luminous bacteria. *Biochim. Biophys. Acta* 5: 175-178.
- Stadler, G. 1906. Ueber das Vorkommen von Leuchtorganen im Tierreich. *Mitt. naturw. Ver. Univ. Wien* 4: 1-16.
- Stammer, H. J. 1930. Das gelegentliche Leuchten der Insekten hervorgerufen durch pathogene Leuchtbakterien. *Mitt. dtsh. ent. Ges.* 1: 38-41.
- Stammer, H. J. 1933. Zur Biologie und Anatomie der leuchtenden Pilzmückenlarve von *Ceroplatus testaceus*. *Daln (Diptera, Fungivoridae)*. *Z. Morph. Oekol. Tiere* 26: 135-146.
- Stammer, H. J. 1935. Das Leuchten des Collembolen, *Achorutes muscorum* Templ., nebst Bemerkungen über die in Deutschland vorkommenden leuchtenden Landtiere. *Biol. Zbl.* 55: 178-182.
- Stebbing, T. R. R. 1888. Amphipoda in Challenger reports, 29. Pp. 1-600 on History.
- Stebbing, T. R. R. 1895. Luminous animals. *Sci. Amer. Supp.* 40: 16595-16596, 16600.
- Steche, O. 1907. Ueber leuchtende Oberflächenfische aus dem malayischen Archipel. *Verh. dtsh. zool. Ges.* 17: 85-92.
- Steche, O. 1908. Beobachtungen über das Leuchten tropischer Lampyriden. *Zool. Anz.* 32: 710-712.
- Steche, O. 1909. Ueber die Leuchtorgane von *Anomalops katoptron* und *Photoblepharon palpebratus*, zwei Oberflächenfischen aus dem malayischen Archipel. Ein Beitrag zur Morph. u. Physiol. der Leuchtorgane der Fische. *Z. wiss. Zool.* 93: 349-408.
- Stedman, J. G. 1805. Eine Bemerkungen über guianische Insekten aus Stedmann's Reise nach Surinam. *Illiger's Mag. Insektenk.* 4: 226-231.
- Stein, F. v. 1883. Der organismus der Infusionstiere. Leipzig, III Abt., 2nd half. Abs. in *J. R. micr. Soc.* (2) 4: 403-404.
- Steinach, E. 1908. Die Summation einzeln unwirksamer Reize als allgemeine Lebenserscheinung. *Pflüg. Arch. ges. Physiol.* 125: 239-346. III. Leuchtzellen von *Lampyrus*, 284-289.
- Stenta, M. 1905. Leuchtorgane bei höheren Tieren. *Verh. zool.-bot. Ges. Wien* 55: 265-266.
- Stephensen, J. 1930. *The Oligochaeta*. Oxford Press, 978 pp. Luminosity, pp. 632-636.
- Stephenson, J. 1914. On a collection of Oligochaeta mainly from Northern India. *Microscolex phosphoreus*. *Records of the Indian Museum*, 10: 321-365. Also in *Nature*, Lond. 94: p. 458.
- Sterne, C. 1897. Das Licht der japanischen Leuchtkäfer. *Prometheus* 9: 6-8, 27-28.
- Sterzinger, I. 1907. Ueber das Leuchtvermögen von *Amphiura squamata* Sars. *Z. wiss. Zool.* 88: 358-384.
- Steuer, A. 1910. *Planktonkunde*. Leipzig and Berlin, 723 pp., pp. 291-334 on Lichtproduction (Meerleuchten) und Lichtperzeption.
- Steuer, A. 1911. *Leitfaden der Planktonkunde*. Leipzig and Berlin, 382 pp. Lichtproduction und Lichtperzeption, 161-186.
- Steuer, A. 1928. Über das sogenannte Leuchtorgan des Tiefsee-Copepoden. *Cephalophanes*. G. O. Sars. *Arb. zool. Inst. Univ. Innsbruck* 3: 9-16.

- Stevens, K. P. 1927. Studies on the amount of light emitted by mixtures of Cypridina luciferin and luciferase. *J. gen. Physiol.* 10: 859-873.
- Stewart, C. 1802. *Elements of the Natural History of the Animal Kingdom*. 2nd ed. 1817. Vol. II, p. 441.
- Stier, A. 1938. Beiträge zur Embryonalentwicklung der *Salpa pinnata*. *Z. Morph. Ökol. Tiere*, 33: 582-632.
- Stillman, W. J. 1883. Glow-worms. *Nature*, Lond. 28: 245.
- Streets, T. H. 1878. The discolored waters of the Gulf of California. *Amer. Nat.* 12: 85-90.
- Strehler, B. L. 1950. Purification and properties of fire-fly luciferesceine. Thesis. John Hopkins Univ. and *Arch. Biochem. Biophys.* 32: 397-406. 1951.
- Strehler, B. L., and W. Arnold. 1951. Light production by green plants. *J. gen. Physiol.* 34: 809-820.
- Strehler, B. L., and W. D. McElroy. 1949. Purification of firefly luciferin. *J. cell. and comp. Physiol.* 34: 457-466.
- Strickland, H. 1834. On the luminosity of the Glow-worm's eggs. *Mag. Nat. Hist. Lond.* 7: 252.
- Strohl, A. 1929. Production de la lumière par les êtres vivants. Biophotogenesis. *In* *Traité de Physiologie normale et pathologique*, ed. by Roger, G. H., and L. Binet Paris, 8: 725-733.
- Stutzer, M. 1929. Über choleraähnliche Vibrionen. *Zbl. Bakt. (Orig.)* 113: 28-35.
- Suchsland, E. 1898. Physikalische Studien über Leuchtbakterien. *Festschr. d. 200-jähr. Jubelfeier der Franckeschen Stiftungen*. 16 pp. Halle, 87. *In* *Zbl. Bakt. (Abt. 2)*, 713-715, 1898.
- Suriray, M. 1836. Recherches sur la cause ordinaire de la phosphorescence marine et description du *Noctiluca miliaris*. *Magazin Zool. Guerin* 6: 1-16.
- Suter, H. 1890. Miscellaneous communications on New Zealand land and fresh water molluscs. *Trans. N.Z. Inst.* 23: 93-96.
- Swinton, A. H. 1880. *Insect variety; its propagation and distribution*. (London, Paris & N.Y.), pp. 87-89.
- Takagi, S. 1933. Mitochondria in the luminous organs of *Watasenia scintillans* (Berry). *Proc. imp. Acad. Japan* 9: 10, 651-654.
- Takagi, S. 1934. Mitochondria in the luminous organs of *Luciola cruciata*. Motschulsky. *Proc. imp. Acad. Japan* 10: 692-694.
- Takagi, S. 1936. Über Sekretbildung in dem Leuchtorgan von *Cypridina hilgendorffii*, Müller, mit besondere Berücksichtigung der Mitochondrien. *Annot. zool. jap.* 15: 344-349.
- Takakuwa, Y. 1941. Eine neue leuchtende *Spirobolellus*-art (Diplopoda) und eine neue *Lamyctes*-art (Chilopoda). *Trans. nat. hist. Soc. Formosa* 31: 84-87.
- Takase, M. 1938. Untersuchungen über den Einfluss von Rohrzucker auf die Leuchtproduktion der Leuchtbakterien. *Sei-i-Kwai med. J.* 57: 1852-1868 (220-236). *In* Japanese with German summary.
- Takase, M. 1939. Untersuchungen über den Einfluss von verschiedenen Salzen auf die Lichtproduktion der Leuchtbakterien. *Sei-i-Kwai med. J.* 58: 54-80. *In* Japanese with German summary.
- Takase, M. 1940. Studies on the action of salts on the spectrum of the luminous bacteria. *Sei-i-Kwai med. J.* 59: 124-142. *In* Japanese.
- Tanassiicuk, N. P. 1925. La structure de l'épithélium glandulaire de *Polycirrus albicans*, Mgn. *Bull. Inst. Sci. Lesshaft.* 11: 109-124. *In* Russian with French summary.

- Tåning, Å. V. 1932. Notes on scopelids from the Dana expeditions. I. Vidensk. Medd. naturhist. Foren. Kbh. 94: 125-146.
- Tarchanoff, J. 1901. Lumière des bacilles phosphorescents de la mer Baltique. C. R. Acad. Sci. Paris 133: 246-249.
- Tarchanoff, J. 1902. Biologisch-chemische Untersuchungen der phosphoreszierenden Bakterien. Zh. med. Khim. 1902, 25-26 (56-74).
- Tarnani, I. G. 1908. Contribution à la question sur la photogénèse chez les Chironomus, Meig.. Rev. russe ent. 8: 87-88. In Russian.
- Tattersall, W. M. 1915. Fauna of the Chilka Lake. The Mysidacea of the lake, with description of a species from the coast of Orino. Mem. Indian Mus. 5: 149-161.
- Taylor, G. W. 1932. The effects of hormones and certain other substances on cell (luminous bacteria) respiration. J. cell. comp. Physiol. 1: 297-331.
- Taylor, G. W. 1934. The effect of narcotics on respiration and luminescence in bacteria, with special reference to the relation between the two processes. J. cell. comp. Physiol. 4: 329-354.
- Taylor, G. W. 1936. The effect of ethyl urethane on bacterial respiration and luminescence. J. cell. comp. Physiol. 7: 409-415.
- Taylor, H. S. 1927. Photochemistry and Chemiluminescence. Bull. nat. Res. Coun. Wash. No. 59, 41-49.
- Terao, A. 1917. Notes on photophores of Sergestes prehensilis, Bate. Annot. zool. jap. 9: 299-316.
- Test, F. C. 1889. New phosphorescent organs in Porichthys. Bull. Essex Inst. 21: 43-52.
- Thesing, C. 1905. Leuchterscheinungen bei Tieren und Pflanzen. Aus d. Natur 1: 353-361.
- Thilo, O. 1910. Leuchtkörper und Scheinwerfer im Tierreich. Natur. Dtsch. öst. naturw. Ges. 1: 167-170.
- Thomas, R. H. 1895, 1902. A luminous centipede. Nature, Lond. 53: 131; 65: 223 (1902).
- Thompson, C. V., and J. Murray. 1885. Rep. Sci. Res. Challenger Exped. Narrative, 1, pt. 2, 743, 935-938.
- Thompson, J. V. 1829. Zoological Researches III. On the luminosity of the ocean, with descriptions of some remarkable species of luminous animals, and particularly of the four new genera, Noctiluca, Cynthia, Lucifer and Podopsis of the Schizopodae, 37-67.
- Thust, K. A. 1916. Zur Anatomie und Histologie der Brisinga coronata G. O. Sars unter besonderer Berücksichtigung der Lumineszenz der Brisingiden. Mitt. zool. Sta. Neapel 22: 367-432.
- Tiedemann, F. 1830. Physiologie des Menschen. 3 vols. Darmstadt. Vol. I, 480-510, Von der Licht-Entwicklung der organischen Körper.
- Tilanus, C. B., Jr. 1888, 1890. Over bacteriën waardoor het lichten van visch veroorzaakt wordt. Versl. Akad. Amst. 4: 117-118 and 7: 238 (1890).
- Tilesius von Tilenau, W. G. 1802. Bemerkungen über einige Quallen oder Meergallerten (Medusa Linn.), welche sich im Tagus und an den Portuguesischen Seeufern finden. Jb. Naturgesch. 1: 166-177.
- Tilesius von Tilenau, W. G. 1819. Leuchten des Meers (arranged by L. W. Gilbert). Gilbert's Ann. Phys. 61: 36-44, 142-160, 161-176, 317-330.
- Tippmar, F. R. 1913. Histologische und vergleichend anatomische untersuchungen an cephalopoden. Z. wiss. Zool. 107: 509-573.

- Tisiro, I. 1937. Über die Lichtproduction des Leuchtköfers; über die Bedeutung der Katalase für die Lichtproduction der Leuchtkäfers. Mitt. med. Akad. Kyoto 21: 66-70, 420.
- Todd, T. J. 1824-1826. An inquiry into the nature of the luminous power of some of the Lampyrides. Quart. Journ. Sci. Lit. Arts 17: 269-270; 21: 241-251. 1926; also in Zool. Journ. 1: 274 (1925).
- Tollhausen, P. 1889. Untersuchungen über *Bacterium phosphorescens* Fischer. Diss. Würzburg.
- Torrey, H. B. 1902. An unusual occurrence of Dinoflagellata on the California coast. Amer. Nat. 36: 187-192.
- Townsend, A. B. 1904. Histology of the light organs of *Photinus marginellus*. Amer. Nat. 38: 127-151. Also in Ann. Transv. Mus. 3: 58-62 and Science N.S. 21: 267, 1905.
- Tozzetti, A. T. 1870. Sull'organo che fa lume nelle luciole volanti d'Italia Centrale (*Luciola italica*) e delle fibre muscolari in questo ed altri insetti ed artropodi. Mem. Soc. ital. Sci. nat. 1: 27.
- Tozzetti, A. T. 1870. Sull'organo che fa lume nelle luciole volanti d'Italia (*Luciola italica*). Nuovo osservat. Boll. Soc. ent. ital. 2: 177-189.
- Treverinus, L. C. 1829. Entwickelt sich Licht und Wärme beim Leben der Gewächse. Tiedeman's Z. Physiol. 3: 257-268.
- Treviranus, G. H. 1802-22. Biologie oder Philosophie der lebenden Natur für Naturforscher und Aerzte. Gottingen 6 vols. Vol. 5, 1818, Zweiter Abschnitt pp. 81-140, Phosphorische Erscheinungen der organischen Natur.
- Treviranus, G. H. 1816. Ueber das Leuchten der *Lampyris splendidula*. Vermischte Schriften anat. u. physiol. Inhaltes, 1: 89-93.
- Trimen, R. 1870. On the occurrence of *Astraptor illuminator*, Murray, or a closely allied insect, near Buenos Ayres. J. linn. Soc. Lond. 10: 503-504.
- Trojan, E. 1906. Neuere Arbeiten über die Leuchtorgane der Fische. Zool. Zbl. 13: 273-284.
- Trojan, E. 1907. Zur Lichtentwicklung in den Photosphären der Euphausien. Arch. mikr. Anat. 70: 177-188.
- Trojan, E. 1909. Leuchtende Ophiopsilen. Arch. mikr. Anat. 73: 883-912; and Biol. Zbl. 28: 343-352, 1908.
- Trojan, E. 1909. Die Lichtentwicklung bei *Amphiura squamata* Sars. Zool. Anz. 34: 776-781.
- Trojan, E. 1910. Ein Betrag zur Histologie von *Phyllirhoë bucephala* Peron u. Lesueur mit besonderer Berücksichtigung des Leuchtvermögens des Tieres. Arch. micr. Anat. 75: 473-517.
- Trojan, E. 1913. Über Hautdrüsen des *Chaetopterus variopedatus*, Clap. S. B. Akad. Wiss. Wien 122: 1-32.
- Trojan, E. 1914. Ueber die Bedeutung der "follicules bacillipares" Claparède's bei "*Chaetopterus variopedatus*." 9th Congr. Internat. Zool., (1913) 390-395.
- Trojan, E. 1914. Das Leuchten und der Farbensinn der Fische. Naturwiss. Wschr. 13: 785-788.
- Trojan, E. 1915. Die Leuchtorgane von *Cyclothone signata*, Garman. S. B. Akad. Wiss. Wien. 124, Abt. 1, 291-316.
- Trojan, E. 1917. Die Lichtentwicklung bei Tieren. Int. Z. phys.-chem. Biol. 3: 94-105. Also: Naturw. Wschr. 16: 457-462.
- Trojan, E. 1929. Die geschlossenen Leuchtorgane der Tiefseefische. Congr. Intern. Zool. à Budapest, Pt. 1, 734-747.

- Trojan, E. 1933. Light-producing powers of sponges. *Nature, Lond.* 131: 728.
- Trotti, L. 1936. Contributo alla conoscenza di probabili organi luminosi nell'*Hymenocephalus italicus* Gigl. *Ann. Mus. Stor. nat. Genova* 59: 160-170.
- Tulasne, L. R. 1848. Sur la phosphorescence spontanée de l'*Agaricus olearius* D.C., du *Rhizomorpha subterranea* Pers et de feuilles mortes du chêne. *Ann. Sci. nat. Bot. (ser. 3)* 9: 338-362.
- Turner, R. D. 1948. The family Tonnidae in the Western Atlantic. *Johnsonia* 2: 165-192.
- Uéno, M. 1938. Stratification of *Noctiluca* in a brackish water lake of Hokkaido. Japan. *Proc. imp. Acad. Japan* 14: 231-232.
- Ugloff, W. A. 1908. Ueber leuchtende Bakterien. *Wojenno-med. J.*, Feb. 1908, 187. Abs. in *Zbl. Bakt. (Abt. I Ref.)* 43: 662 (1909).
- Usami, S., and M. Yokoseki. 1948. On the effect of certain substances upon light production of a luminous bacterium. *Seibutu* 3: 210-217. In Japanese, with English summary.
- Ussow, M. 1879. Ueber den Bau der sogenannten augenähnlichen Flecken einiger Knochenfische. *Bull. Soc. Nat. Moscou* 54: 79-115.
- Vailliant, L. 1886. Poissons. *Exped. Sci. du Talisman and Travailleur 1880-1883*, 406 pp. Paris.
- Vallentin, R., and J. T. Cunningham. 1888. The photospheria of *Nyctiphanes Norvegica*. *Quart. J. micr. Sci. N.S.* 28: 318-341.
- Van Beneden, P. 1846. Rapport sur le memoire du docteur Verhaeghe, ayant pour titre: Recherches sur la cause de la phosphorescence de la mer dans les parages d'Ostende. *Bull. Acad. Belg.* 13: (2nd part), 3-17.
- Van der Burg, A. 1943. Spektrale onderzoekingen over chemo- en bioluminescentie. Thesis. Utrecht, 107 pp. In Dutch.
- Van der Kerk, G. J. M. 1942. Onderzoekingen over de bioluminescentie der lichtbacterien. Thesis. Utrecht, 161 pp. In Dutch with English and German summaries.
- Van de Rovaart, H. 1910. Lichtgevende bacterien. *Handel v. h. XIV vlaamsch. nat.-en geneesk. Cong. Gent.*, 197-202.
- Vanhöffen, E. 1895. Das Leuchten von *Metridia longa* Lubbock. *Zool. Anz.* 18: 304-305.
- Van Lummel, L. A. E. 1932. Over lichtende wormpjes in de baai van Batavia. *Trop. Natuur.* 21: 85-87.
- Van Schouwenburg, K. L. 1938. On respiration and light emission in luminous bacteria. Thesis. Delft, Holland. 97 pp.
- Van Schouwenburg, K. L. 1940. On the catalase content of luminous bacteria. *Enzymologia* 8: 344-352.
- Van Schouwenburg, K. L., and J. G. Eymers. 1936. Quantum relationship of the light-emitting process of luminous bacteria. *Nature, Lond.* 138: 245.
- Van Schouwenburg, K. L., and A. Van der Burg. 1940. On the influence of carbon monoxide on respiration and light emission of luminous bacteria. *Enzymologia* 9: 34-42.
- Van Vollenhoven, S. C. S. 1860. *Natuurlijke Historie van Nederland*. Haarlem.
- Vejdovsky, F. 1878. Beiträge zur Kenntnis der Tomopteriden. *Z. wiss. Zool.* 31: 81-100.
- Vejdovsky, F. 1884. Über die Phosphorescence der Regenwürmer. *System und Morphologie der Oligochaeten*. Prague, 1884, p. 67.

- Vélain, C. 1877. Observations general sur la faune des deux îles suivies d'une description des mollusques. Arch. zool. exp. gén. 6: 1-144.
- Verany, J. B. 1851. Mollusques mediterraneens. I. Cephalopods de la Méditerranée, Genes, 116.
- Verhaeghe. 1848. Recherches sur la cause de la phosphorescence de la mer, dans les parages d'Ostende. Mém. Sav. étr. Acad. Sci. Belg. 22: 31 pp.
- Verhoeff, K. W. 1908. Chilopoda, Leuchtvermögen. In Bronns Klassen 5 (2) Lief. 80-82, 310-312.
- Verhoeff, K. W. 1924. Zur Biologie der Lampyriden. Z. wiss. InsektBiol. 19: 79-88, 122-127, 135-143.
- Verrill, A. E. 1884. Evidences of the existence of light at great depths in the sea. Nature, Lond. 30: 280-281.
- Verworn, M. 1892. Ein automatisches Centrum für die Lichtproduktion bei *Luciola italica*, L. Zbl. Physiol. 6: 69-74.
- Vessicelli, N. 1906. Contribuzioni allo studio della *Phylliroë bucephala* Peron and Lesueur. Mitt. zool. Sta. Neapel 18: 105-135.
- Vessicelli, N. 1910. Nuove Contribuzioni allo studio della *Phylliröe bucephala* Peron and Lesueur. Mitt. zool. Sta. Neapel 20: 108-128.
- Vignal, W. 1878. Researches histologiques et physiologiques sur les noctiluques (*Noctiluca miliaris*, Suriray). Arch. Physiol. Norm. Pathol. (Ser. 2) 5: 415-454.
- Villiers. 1842. Note sur la propriete phosphorescente de petites Fourmis jaunes. Ann. Soc. ent. Fr. 11, Bull., xiii-xiv.
- Vivanti, A. 1914. Contributo alla cognoscenza dei Cefalopodi abissali del Mediterraneo. Ricerche sulla *Carybditeuthis maculata* n.sp. dello Stretto di Messina. Arch. zool. ital. Napoli 7: 55-79.
- Viviani, D. 1805. Phosphorescentia maris. Quattuordecim lucescentium animalorum novis speciebus illustrata. Geneva, 17 pp.
- Vogel, R. 1912. Beiträge zur Anatomie und Biologie der Larve von *Lampyris noctiluca*. Zool. Anz. 39: 515-519.
- Vogel, R. 1913. Zur Topographie und Entwicklungsgeschichte der Leuchtorgane von *Lampyris noctiluca*. Zool. Anz. 41: 325-332.
- Vogel, R. 1915. Beitrag zur Kenntnis des Baues und der Lebensweise der Larve von *Lampyris noctiluca*. Z. wiss. Zool. 112: 291-432.
- Vogel, R. 1921. Bemerkungen zur Topographie und Anatomie der Leuchtorgane von *Luciola chinensis*, L. Jena. Z. Naturw. 57: 269-274.
- Vogel, R. 1922. Über die Topographie der Leuchtorgane von *Phausis splendidula* Leconte. Biol. Zbl. 42: 138-140.
- Vogel, R. 1927. Lampyrinae. Leuchtkäfer. In Biol. Tiere Dtschl. 11g. 21. 30: 382-391.
- Vonwiller, P. 1920. Anatomische Bemerkungen über den Bau der Leuchtorgane von *Lampyris splendidula*. Festschr. f. Zschokke, No. 34, Basel, 7 pp.
- Voormolen, C. M. 1918. Über den Einfluss der Strahlung von Mesothorium und Polonium auf das Wachstum der Leuchtbakterien. Rec. Trav. bot. neerland. 15: 229-237.
- Vorderman, A. G. 1900. Twee lichtgewende visschen van Banda. Ikan lewéri batoo (*Heterophthalmus palpebratus* Lac.) en Ikan lewéri aier (*Heterophthalmus katoptron* Blkr.). Natuurk. Tijdschr. Ned.-Ind. 59: 72-77.
- Vouk, V., V. Skoric and Z. Klas. 1931. A new phosphorescent bacterium from

- the Adriatic Sea and the pH range of its luminosity. Abstracted from "Rad." 241: 229-238. Bull. int. Acad. yougoslave Sci. 25: 86-88.
- Wahlberg, P. 1849. Merkwürdiger Instinkt und Lichtentwicklung bei einer schwedischen Mückenart (*Ceroplatus sesiodes*). From Oversigt K. Vetenskaps Akad. Förh. Stockholm (1848), 5: 128-131 and Stettin. ent. Ztg. 10: 120-123.
- Walter, A. 1909. Das leuchten einer terrestrischen Oligochäte. Trav. Soc. Nat. St.-Pétersb. 40: 103-9; in Russian with German resume, 136-137.
- Warbasse, W. W. and F. H. Johnson. 1950. The influence of penicillin on large body production by luminous bacteria. J. Bact. 60: 279-282.
- Warren, G. H. 1945. The antigenic structure and specificity of luminous bacteria. J. Bact. 49: 547-561.
- Wasmann, P. E. 1896. Natur und Zweck des thierischen Leuchtvermögens. Insektenbörse 13: 95-96, 101-102, 110-111, 117-118.
- Wassink, E. C. 1948. Observations on the luminescence in Fungi, I, Including a critical review of the species mentioned as luminescent in literature. Rec. Trav. bot. néerland. 41: 150-212.
- Watanabe, H. 1897. The phosphorescence of *Cypridina hilgendorfi*. Annot. zool. jap. 1: 69-70.
- Watasé, S. 1895. On the physical basis of animal phosphorescence. Biol. Lect. Marine Biol. Lab., Wood's Hole, 101-119.
- Watasé, S. 1898. Protoplasmic contractility and phosphorescence. Biol. Lect. Marine Biol. Lab. Wood's Hole, 177-192.
- Watasé, S. 1905. (On the luminous organs of *Abraliopsis*) Dobutugaku Tassi. Zool. Mag. Tokyo. 17: 119-122. In Japanese.
- Waterhouse, C. O. 1889. (Larva (?) of *Phengodes*.) Trans. R. ent. Soc. Lond. 1889, Proc. p. 30.
- Waterman, T. H. 1939. Studies on deep-sea angler-fishes (Ceratoidea). I. An historical survey of our present state of knowledge. II. Three new species. Bull. Mus. comp. Zool. Harv. 85: 65-94.
- Waterman, T. H. 1948. Studies on deep-sea angler fishes. III. The comparative anatomy of *Gigantactis longicirra* Waterman. J. Morph. 82: 81-150.
- Waterman, T. H., R. F. Nunnemacher, F. A. Chace and G. L. Clark. 1939. Diurnal vertical migrations of deep-water plankton. Biol. Bull. Wood's Hole 76: 256-279.
- Waterton, C. 1871. Essays on Natural History, chiefly Ornithology. London, p. 594 in appendix.
- Webb, W. 1855. On the *Noctiluca miliaris*. Quart. J. micr. Sci. 3: 102-106.
- Weber, M. 1902. Siboga-Expeditie. Introduction et description de l'expédition Leyden, 108-110. (German Ed., Leipzig, 1905.)
- Weber, M. 1913. Die Fische der Siboga-Expedition. Siboga-Expeditie LVII Leiden.
- Weber, M. and A. Weber-Van Bosse. 1890. Quelques nouveaux cas de Symbiose. Symbiose du *Noctiluca miliaris* avec une Algue unicellulaire verte (pp. 69-71). Zool. Ergeb. einer Reise in Niederländisch Ost-Indien. Leiden 1: 48-71.
- Webster. 1834. Über Medusen und das Leuchten derselben. Fries's Not. Geb. Natur und Heilk. 42: 247-8.
- Weill, R. 1929. Protistologica XVI. Observation sur la symbiose entre Noctiluques et Algues vertes. Archives Zool. exp. gén. 69 "Notes et Revue," 13-21.
- Weill, R. 1938. Statistiques et hypothèses sur les organes lumineux des poissons bathypelagiques. Rev. sci. Paris 76: 283-287.

- Weitlaner, F. 1902. Tagebuchnotizen eines Schiffsarztes über das Meersleuchten. Verh. zool.-bot. Ges. Wien, 52: 270-277.
- Weitlaner, F. 1909. Etwas vom Johannis-Käferlicht. Verh. zool.-bot. Ges. Wien, 59: 94-103.
- Weitlaner, F. 1911. Weiteres vom Johanniskäferchenlicht, und vom Organismenleuchten überhaupt. Verh. zool.-bot. Ges. Wien, 61: 192-202. Review in Zbl. Biochem. Biophys. 12: 351.
- Weleminsky, F. 1895. Die Ursachen des Leuchtens bei Choleravibrionen. Prager med. Wschr. 20: 263-264.
- Welsh, J. H. 1938. Diurnal rhythms. Quart. Rev. Biol. 13: 123-139. See p. 124.
- Welsh, J. H., and F. A. Chace, Jr. 1937, 38. Eyes of deep sea crustaceans. I. Acanthephyridae. II. Sergestidae. Biol. Bull. Wood's Hole 72: 57-74; 74: 364-375, 1938.
- Welsh, J. H., F. A. Chace, Jr. and R. F. Nunnemacher. 1937. The diurnal migration of deep-water animals. Biol. Bull. Wood's Hole 73: 185-196.
- Wenig, K. 1946. The chemical and physical basis of the luminescence of the earthworm, *Eisenia montana*. Vestn. cs. Zool. Spol. 10: 293-359.
- Wenig, K., and V. Kubista. 1949. The presence of riboflavin in the luminous material of the earthworm *Eisenia submontana*. Experientia 5: 73.
- Wenzel, H. W. 1896. Notes on Lampyridae with the description of a female and larva. Ent. News 7: 294-296.
- Werneck. 1841. Untersuchungen über mikroskopische Organismen in der umgebung von Salzburg. Mber. Berlin Akad. Wiss. 102-110; 373-377.
- Wesmaël, C. 1837. Phosphorescence du Fulgore porte-lanterne. Ann. Soc. ent. Fr. 6, Bull. 67.
- Wesmael, C. 1838. Note sur la Fulgore Lanterne. Bull. Acad. Belg. 4: 136.
- Westwood, J. O. 1831. Further remarks on the luminosity of the sea. Mag. Nat. Hist. 4: 505-511.
- Westwood, J. O. 1839. On the family Fulgoridae, with a monograph of the genus Fulgora of Linnaeus. Trans. Linn. Soc. Lond. 18: 133-153.
- Westwood, J. O. 1854. Luminosity of *Helobia brevicollis*. Trans. R. ent. Soc. Lond., proc. 34. Also in: Zoologist (1855) 13: 4565.
- Weyenbergh, H. 1876-1877. Eine leuchtende Käfer-larve. Horae Soc. ent. ross 12: 177-180.
- Wheeler, W. M. 1916. A phosphorescent ant. Psyche, Cambridge, Mass. 23: 173-174.
- Wheeler, W. M., and F. X. Williams. 1915. The Luminous organ of the New Zealand glow-worm. Psyche, Cambridge, Mass. 22: 36-43.
- White, A. 1844. Description of some new species of Coleoptera and Hemiptera from China. Pp. 422-426 quotes Bowring.
- White, W. H. 1835. The glow-worm; the results of experiments in elucidation of a knowledge of its habits. Mag. Nat. Hist. 8: 623-625.
- Wied-Neuwied, M. von. 1820-21. Reise nach Brasilien in den Jahren 1815 bis 1817, 2 vols. Frankfurt a.M., 2: 111.
- Wielowiejski, H. R. von. 1882. Studien über die Lampyriden. Z. wiss. Zool. 37: 251-258.
- Wielowiejski, H. R. von. 1889. Beiträge zur Kenntnis der Leuchtorgane der Insekten. Zool. Anz. 12: 594-600.
- Wielowiejski, H. R. von. 1890. Contributions a l'histoire des organes lumineux chez les insectes. Bull. Sci. Fr. Belg. 28: 145-207.

- Wigglesworth, V. B. 1949. The light of Glow worms and Fire flies. *Sci. News*, 12, July.
- Will, F. 1844. Über das Leuchten einige Seethiere. *Arch. Naturgesch.* 10: 328-337. Also in *Horae tergestinae*, pp. 57-58.
- Williams, F. X. 1914. Abnormal larva of *Photuris pennsylvanica*. *Psyche*, Cambridge, Mass., 21: 126-129.
- Williams, F. X. 1916. Photogenic organs and embryology of Lampyrids. *J. Morph.* 28: 145-186.
- Williams, F. X. 1917. Notes on the life-history of some North American lampyridae. *J. N. Y. ent. Soc.* 25: 11-33.
- Williamson, K. B. 1948. Note on luminous dytiscid beetle. *Nature*, Lond. 162: 768.
- Wolff, M. 1907. Das Licht in der Tiefe des Weltmeeres. *Nat. Wschr.* 32: (N.F. 6), 355-558.
- Woltereck, R. 1905. Scypholanceola, eine neue Hyperidengattung mit Reflektororgan. *Zool. Anz.* 29: 413-416.
- Wood, R. W. 1939. A fire-fly "Spinthariscopes." *Science* 90: 233-234.
- Yamada, T. 1937. On the spawning of the squid, *Watasenia scintillans* in the waters off the east coast of Tyôsen. *Bull. Jap. Soc. Sci. Fish.* 6: 75-78.
- Yamagata, S., and H. Nakamura. 1937-1938. Ueber die Hydrogenase des einer Bemerkung über den mechanismus der bakteriellen Knallreaktion. *Acta Phytochim*, Tokyo 10: 297-311.
- Yasaki, Y. 1927. Bacteriologic studies on bioluminescence. I. On the Cause of luminescence in the fresh water shrimp, *Xiphocaridina compressa* (De Haan). *J. infect. Dis.* 40: 404-407.
- Yasaki, Y. 1928. On the nature of the luminescence of the knight fish, *Monocentris japonicus* (Houttuyn). *J. exp. Zool.* 50: 495-505.
- Yasaki, Y. 1929. On the so-called symbiotic bioluminescence. *Zool. Mag. (Tokyo)* 41: 490-491.
- Yasaki, Y. 1943. Studies on bioluminescence. *J. Physiol. Jap.* 253-268. In Japanese.
- Yasaki, Y., and Y. Haneda. 1935. Bioluminescence of the deep-sea fish, *Macrouridae*. *J. Appl. Zool. Tokyo* 7: 165-176. In Japanese.
- Yasaki, Y., and Y. Haneda. 1935. Über einen neuen Typus von Leuchtorgan im Fische. [*Acropoma japonicum*.] *Proc. imp. Acad. Japan* 12: (2), 55-57.
- Yasaki, Y., M. Nishio, A. Ichikawa, R. Magima, and O. Ishikawa. 1926. Bacteriological studies on bioluminescence. II. On the nature of the new luminous bacteria, *Microspira phosphoreum* Yasaki. *Sei-i-Kwai med. J.* 45: (3). In Japanese.
- Yatsu, N. 1912. Observations and experiments on the Ctenophore egg: I. The structure of the egg and experiments on cell-division. *J. Coll. Sci. Tokyo* 32: art. 3, 21 pp.
- Yatsu, N. 1917. Note on the structure of the maxillary gland of *Cypridina hilgendorffii*. *J. Morph.* 29: 435-440.
- Yosida, K., T. Nakamura, and T. Okada. 1935. Über die Lichtproduction des Leuchtkäfers. I. *Mitt. wed. Akad. Kioto* 15: 1149. Abstract in German.
- Young, C. A. 1870. The spectrum of the fire-fly. *Am. Nat.* 3: 615; also in *J. R. Soc. Arts. and Trans. R. ent. Soc. Lond. Proc.* p. 17.
- Zacharias, O. 1905. Beobachtungen über das Leuchtvermögen von *Ceratium*

- tripos (Müll.). Biol. Zbl. 25: 20-30. Also in Forsch. Ber. biol. Sta. Plön. 12: 316-330.
- Zikes, H. 1913. Ueber das Verhalten von Leuchtenbakterien in Würze und Bier. Allg. Z. Bierbrau. 40: No. 7. Abs. in Zbl. Bakt. (Abt. 2), 37: 88, 1913.
- Zirpolo, G. 1917. Recherche su di un bacillo fosforescente che si svilluppa sulla *Sepia officinalis*. Boll. Soc. Nat. Napoli 30: 47-77.
- Zirpolo, G. 1918. I Batteri fotogeni degli organi luminosi di *Sepiola intermedia*. Naef. (Bacillus Pierantonii n. sp.). Bull. Soc. Nat. Napoli 30: 206-220.
- Zirpolo, G. 1918. Micrococcus Pierantonii, nuova specie di batteria fotogeno dell'organo luminoso di Rondoletia minor, Naef. Boll. Soc. Nat. Napoli 31: 75-87.
- Zirpolo, G. 1919. I batteri fosforescenti e la recenti ricerche sulla biofotogenesi. Natura. Milano 10: 60-72.
- Zirpolo, G. 1920. Studi sulla bioluminescenza batterica. I. Azione degli ipnotici. Riv. Biol., 2: 52-59.
- Zirpolo, G. 1920. Studi sulla bioluminescenza batterica. II. Azione dei sali magnesica. Boll. Soc. Nat. Napoli 32: 112-119.
- Zirpolo, G. 1920. Studi sulla bioluminescenza batterica. III. Azione dei raggi emanati dal bromuro di radio. Boll. Soc. Nat. Napoli 33: 75-81.
- Zirpolo, G. 1921. Studi sulla bioluminescenza batterica. IV. Azione dei sali radioattivi. Riv. Sci. Nat. Napoli 12: 139-144.
- Zirpolo, G. 1922. Studi sulla bioluminescenza batterica. VI. Azione dei sali di chinina, caffeina cocaina e strichnina. Riv. Sci. Nat. Napoli 13: 1-11.
- Zirpolo, G. 1922. Studi sulla bioluminescenza batterica. V. Azione del nitrato di cerio. Boll. Soc. Nat. Napoli 34: 46-50.
- Zirpolo, G. 1922. Osservazioni sulla biofotogenesi. Boll. Soc. Nat. Napoli 34: 128-132.
- Zirpolo, G. 1923. Ricerche sulla simbiosi tra Zoaxantelle e *Phyllirohoe bucephala*. Boll. Soc. Nat. Napoli 35: 129-138.
- Zirpolo, G. 1923. Studi sulla bioluminescenza batterica. VII. Azione dei sali di potassio. Boll. Soc. Nat. Napoli 35: 245-247.
- Zirpolo, G. 1924, 1926. Ancora sui batteri fotogeni. Riv. Biol. 4: 1-4; 8: 244-248.
- Zirpolo, G. 1927. Studi sulla bioluminescenza batterica. VIII. La resistenza del potere luminoso. Boll. Soc. Nat. Napoli 18: 225-231.
- Zirpolo, G. 1929. Studi sulla bioluminescenza batterica. IX. Azione delle alte e basse temperature sui batteri luminosi. Boll. Soc. Nat. Napoli 41: 137-149.
- Zirpolo, G. 1931. Studi sulla bioluminescenza batterica. X. Azione dei batteri luminosi sulle germinazione dei semi. Boll. Soc. Nat. Napoli 43: 393-422.
- Zirpolo, G. 1932. Studi sulla bioluminescenza batterica. XI. Batteri luminosi ed "Anelli di Liesegang." Boll. Soc. Nat. Napoli 44: 221-228.
- Zirpolo, G. 1932. Studi sulla bioluminescenza batterica. XII. Azione dell'idrogeno (-253°C.) e dell'elio liquido (-269°C.). Boll. Soc. Nat. Napoli 44: 229-235.
- Zirpolo, G. 1933. Ricerche criobiologiche sui batteri luminosi dei Cefalopodi. Arch. zool. (ital.) Napoli 18: 359-405.
- Zirpolo, G. 1938. Studi sulla bioluminescenza batterica. XIII. Azione dell'acqua pesante (D_2O). Boll. Zool. 9: 49-55.
- Zopf, W. 1890. Die Pilze in morphologischer, physiologischer, biologischer und systematischer Beziehung. Breslau.
- Zugmayer, E. 1910. Leuchtorgane und Augen von Tiefseefischen. Nat. Wschr. 25: 329-331.
- Zugmayer, E. 1911. Poissons. Result. Camp. sci. Monaco Fasc. 35, pp. 1-174.

Subject Index

All generic and family names of luminous or allegedly luminous animals and plants are included in the index. Groups of non-luminous animals and non-luminous genera in the classifications and geographical locations or names of vessels of expeditions have not been indexed. Chemicals of special interest are indexed separately while others are included as groups of compounds. For example, glucose, sucrose, etc., will be found under sugars and NaCl, K₂SO₄, CaCl₂, etc., under salts rather than as separate entities. References of special interest are in bold-face type. The paging of sections will be found in the table of contents.

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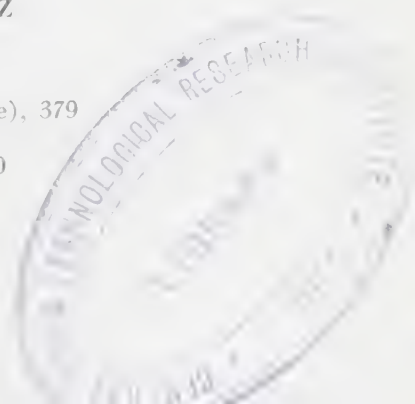
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